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Effects of Olfactory Enrichments on African Cheetahs (Acinonyx Jubatus)

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EFFECTS OF OLFACTORY ENRICHMENT ON AFRICAN CHEETAHS (*ACINONYX JUBATUS*)

by

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B.A., Southern Illinois University, 2008

A Thesis
Submitted in Partial Fulfillment of the Requirements for the
Master of Science

Department of Zoology
in the Graduate School
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THESIS APPROVAL

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Marcus Abston

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in the field of Zoology

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Environmental enrichment has been an essential part of felid husbandry in zoos, serving to reduce both physiological and psychological stress. Olfactory enrichment is one of many interventions used to prevent stereotypic behavior caused by stress in felids. However, little research has been done on this practice. The purpose of this study was to compare behavioral responses of four captive African cheetahs to six types of commercial fragrances used as olfactory enrichment stimuli. Three of these fragrances are marketed as “men’s cologne” and three as “women’s perfume”. The fragrances were also categorized as musky, spice, and floral types. Behavioral responses were recorded by live observations during 36 bouts at the St. Louis Zoo. There was a significant difference between cheetahs’ interaction time with male and female fragrances; they seemed to prefer male colognes. However, there was no significant difference in interaction time among musky, spice, and floral fragrance types. Engagement behaviors varied among individuals (e.g. sniffing, scent rolling, pawing), but were similar to those reported by previous authors. These findings suggest that commercial fragrances may be a useful option for African cheetah olfactory enrichment.

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CHAPTER 1

INTRODUCTION

The focus of zoos altered in the 1960s as zoo biologists became more aware of the importance of ensuring the survival of threatened species (Ballantyne et al. 2007). This awareness prompted zoos to become institutions with a significant role in wildlife conservation during the early 1980s, instead of being focused on entertainment (Tribe and Booth 2003, Miller et al. 2004, Ballantyne et al. 2007). Ex situ conservation in the form of captive breeding programs has become a main focus of many zoos, producing captive populations that may eventually replenish and increase the genetic diversity of wild populations (Hanks 2001, Tribe and Booth 2003). Hanks (2001) noted that ex situ programs are especially promising for threatened species with which zoos have husbandry experience. The development of effective breeding programs requires understanding fundamental reproductive traits and basic species biology, and has prompted much research in these areas (Swanson et al. 2003). Predatory mammals, especially felids, are the most common type of zoo animals and have been the focus of numerous captive-breeding initiatives, several of which have been successful (Quirke and O' Riordan 2011). Species with realistic prospects for reintroduction into the wild are the primary targets, provided that high-quality habitat (wherein threats to the species concerned have been minimized) is available (Hanks 2001). Many zoos now manage breeding programs cooperatively, with multiple institutions working to improve the reintroduction potential of threatened species (Tribe and Booth, 2003, Ballantyne et al. 2007).

The majority of knowledge about felid reproductive biology vital to management and conservation has been gained from captive animals. Law et al. (1997) and Brown (2011) noted that studies on captive felids have assisted reproductive research by demonstrating the effects of photoperiod and season on felid breeding preferences. Indeed, numerous biological and management-related factors have been shown to affect the reproductive success of captive cats (Beekman et al. 1999, Saunders et al. 2014). One of the most widely cited biological factors is female age, but its influence on offspring survival is unclear (Longley 2011, Saunders et al. 2014). Longley (2011) stated that decline in female fertility with age is a great concern for felid management, but males remain reproductively useful longer than females. Saunders et al. (2014) noted that staff experience and management protocols also impact captive breeding success. Several studies suggest that continued exposure to potential mates promotes successful reproduction; others have observed that sheltering cats together has a negative effect and that prospective breeding males should only be introduced during female estrus (Saunders et al. 2014). However, it is difficult to recognize when a particular female has entered estrus (Brown, 2011). Factors such as litter size, degree of inbreeding, and parental age are predictors of cub survival in African cheetahs (*Acinonyx jubatus*) (Beekman et al., 1999; Saunders et al. 2014). The most significant obstacle to captive breeding success, however, is the stress of captivity, and an extensive literature has developed on this topic.

Stereotypical Behaviors And Stress Related Health Effects

Negative Effects of Stress. Stress can lead to serious welfare problems in captive animals (McPhee 2002, Swanson and Brown 2004, Clubb and Vickery 2006, Hope and Deem 2006, Gouveia et al. 2011). An animal's welfare is difficult to measure because it is defined by a combination of physiological, behavioral, and biochemical factors (Bassett and Buchanan-Smith 2007). Broom and Ferguson (2007), Stella et al. (2013), and the American Veterinary Medical Association (2015) defined welfare as the positive state of an animal and its ability to cope with its environment, indicated by whether it is well nourished, healthy, able to express natural behavior, comfortable, safe, and not suffering from distress, pain, or fear. It is uncontroversial among animal care practitioners that prolonged stress has a negative impact on welfare.

The stress response normally involves the release of adrenal glucocorticoids (GCCs, chiefly cortisol) and catecholamines, as well as suppression of hormones related to anabolism, reproduction, and growth (Morgan and Tromberg 2007, Farooq et al. 2011). Cortisol levels are often used as an indicator of stress though they are also affected by age, individuality, and season (Wilson et al. 2004, Fazio et al. 2008, Iki et al. 2011, Bechshøft et al. 2012, Fureix et al. 2013). Iki et al. (2011) noted that cortisol is released naturally through activation of the hypothalamic-pituitary-adrenal (HPA) axis to help animals cope with unexpected circumstances (“stressors”). The biological effects of stress depend on the duration of the physiological stress response (Dhabhar 2009). Acute stress can last for minutes to hours, and can be repeated at different frequencies or durations. Acute stress typically is manifested by the "fight-or-flight" response and is the mechanism that incites musculoskeletal, neuroendocrine, and cardiovascular systems into action for survival. Acute stress is not necessarily damaging as long as the physiological mediators return to resting levels soon after its termination. Acute stress may facilitate or even be necessary for reproductive activation in certain species. An anecdotal account of acute stress correlated with reproductive activation involved a pair of cheetahs that were given live chickens for food; the excitement led to the pair’s first mating attempt (Carlstead and Shepherdson 1994). In contrast, chronic stress continues for several hours per day for weeks or months (Dhabhar 2009).

Chronic stress has negative physiological and behavioral consequences. Behavioral indications include pacing, lethargy, head bobbing, swaying, constant grooming, chewing on cage bars, tendency to freeze, intensified vigilance, increased hiding, reduced behavioral complexity, aggressiveness, and reduced ability to cope (Lyons et al. 1997, Jenny and Schmid 2002, McPhee 2002, Wilson et al. 2004, Morgan and Tromberg 2007, Meagher et al. 2012). Reduction in explorative and reproductive behavior, poor maternal care, diminished social interactions, and depression-like states also result from chronic stress (Clubb and Vickery 2006, Farooq et al. 2011, Meagher et al. 2012). In addition, exhibits that are too small or lack natural substrates lead to increased stress levels and associated changes in cortisol secretion (Law et al. 1997, Lyons et al. 1997, Swanson and Brown 2004, Farooq et al. 2011). In felids, stress causes exploratory and play behaviors to be reduced and replaced by alert behavior and extra time awake (Morgan and Tromberg 2007, Iki et al. 2011). Sickness behavior in felids is also linked to environmental stressors (Stella et al. 2013). Sickness behavior refers to a group of nonspecific clinical symptoms (e.g. anorexia, defecating out of the litter pan, lethargy, vomiting, decreased food and water intake, fever, pain displays, somnolence, diarrhea). These symptoms inhibit normal activities such as social contact, grooming, or feeding, perhaps as a means to conserve energy for enhanced immune function (Stella et al. 2011, 2013, 2014).

Physiological consequences of long-term stress are mediated by hormone imbalances. For example, opioids regulate several endocrine pathways that are involved in responses to stress, and disruption of these pathways may result in early mortality (Morgan and Tromberg 2007, Fazio et al. 2008) or male-biased litters (Faust and Thompson 2000). Stressed animals show increased β-endorphin levels, glucose metabolism, respiration rate, and heart rate (Morgan and Tromberg 2007, Fazio et al. 2008). Stressors may also decrease growth hormone levels resulting in lower growth rate, reduced body weight, increased mortality/morbidity rates, and suppressed reproductive cycling (Carlstead and Shepherdson 1994, Clubb and Vickery 2006, Mason et al. 2007, Morgan and Tromberg 2007, Fureix et al. 2013, Chebaani et al. 2014).

Prolonged high levels of cortisol and other circulating GCCs damage areas of the brain responsible for stopping the stress response. An extended upsurge of GCCs can become self-sustaining, as an increase in numerous isomers of GCCs shifts metabolism toward energy mobilization and away from energy conservation, and also suppresses the immune system (Morgan and Tromberg 2007, Fazio et al. 2008, Farooq et al. 2011, Iki et al. 2011, Thoma 2011, Bechshøft et al. 2012, Stella et al. 2013). The enhanced delivery of fatty acids, glucose, and triglycerides to skeletal muscles and the brain by means of hepatic glucose release and visceral lipolysis constrains immune responses (Farooq et al. 2011). Research has indicated that reduced availability of tryptophan for serotonin production occurs as stress reroutes tryptophan to vitamin B_3 synthesis (Thoma 2011). Reduced serotonin can disrupt sleep by reducing nighttime availability of melatonin, and sleep deprivation negatively affects the immune system (e.g. disrupts T-cell function) (Lange et al. 2010, Thoma 2011). Decreased immune function may occur in the intestinal system as well as in lymphoid tissue (Niklasson et al. 2014).

Numerous physiological conditions that are connected to stressors have been documented in felids. McPhee (2002) noted that dental problems such as plaque formation and palatine erosion may occur in felids that experience prolonged stress. Moreover, high levels of β-endorphin can stimulate excessive scratching (Willemse et al. 1994). Stress-induced physiological changes in felids include increased rectal temperature, heart rate, blood pressure, and respiratory rate (Lockhart et al. 2013; Nibblett et al. 2014). The utility of a blood sample as a reliable health marker can be significantly compromised by stress-induced hormonal changes (Lockhart et al. 2013; Nibblett et al. 2014). Pro-inflammatory intestinal difficulties in felids are associated with stressors; interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and tumor necrosis factor alpha (TNF-α) are alleged to play a significant part in the expression of sickness behavior

(Stella et al. 2013). Cats with depression or inflammatory diseases (e.g. cancer, cardiovascular disease, rheumatoid arthritis) have increased peripheral cytokine concentrations (Stella et al. 2013). Carlstead and Shepherdson (1994) noted that domestic cats (*Felis catus*) had compromised reproductive function when experiencing chronic stress.

Stress and Stereotypic Behaviors. One of the consequences of stress in captive mammals is stereotypic behavior, actions that are rigidly repeated with no apparent function or goal (Lyons et al. 1997, Wooster 1997, Wilson et al. 2004). The exact causes of stereotypic behaviors are uncertain, but their management has become a critical concern in captive animal husbandry (Rushen and Mason 2006, Fureix et al. 2013). Stereotypies typically increase when animals experience stressful situations and their welfare has been compromised (Wilson et al. 2004, Gilbert and Baker 2011). Early studies on stereotypical behavior were descriptive, but more recent work focuses on causes (Rushen and Mason 2006). Although a correlation between stereotypic behavior and poor welfare has been demonstrated, individuals of similar welfare status often vary in their level of stereotypy due to age, social history and season of year (Rushen and Mason 2006, Wilson et al. 2004; Iki et al. 2011). The frequency of stereotypic behavior is also affected by repeated transportations, enclosure type, scheduling of care events, and type of restraint (Wilson et al. 2004, Rushen and Mason 2006, Morgan and Tromberg 2007). Greater understanding of the cause of stereotypic behaviors could lead to husbandry practices that prevent them and reduce the stress of captivity (Rushen and Mason 2006).

Carnivorans are especially disposed to stereotypical behavior in zoo settings, though research has focused less on the motivational bases of behaviors than on their prevention and reduction (Clubb and Vickery 2006). It is recognized that predatory mammals need positive stimulation in zoo habitats to promote species-typical behavior such as natural foraging and exploration (Powell 1997, Wooster 1997). For this reason, research on stereotypic behavior in carnivores has centered on how stereotypies change in response to modifications of the enclosure environment and to explain differences in pacing behavior among species using the comparative method (Clubb and Vickery 2006). Locomotory stereotypies are the most common type among carnivorans and appear to result from lack of foraging opportunities. An example of a locomotory stereotype is pacing (Krawczel et al. 2005, Clubb and Vickery 2006). Pacing in captive carnivores is correlated with size of enclosure, degree of dietary specialization for carnivory, breeding season, size of natural home range, natural caching behavior, and regularity of daily feeding times (Clubb and Vickery 2006, Farooq et al. 2011). Thus, the display of similar stereotypical behaviors in carnivores may be triggered by different causes.

Some studies have shown that changes in food ratios have an effect on stereotypic behavior in carnivores. Variations to food presentation (e.g. swing pole feeders, scatter feeding on the ground, food poles) is the most common form of environmental enrichment for carnivores, allowing animals to perform natural foraging behaviors (Law et al. 1997, Clubb and Vickery 2006). These methods have resulted in decreased locomotory stereotypies, but are not universally successful (Clubb and Vickery 2006). Wide-ranging carnivores may develop stereotypic behavior as a result of being in small enclosures, but there has been little research on this connection (Swaisgood and Shepherdson 2005, Clubb and Vickery 2006). Stereotypic behaviors often occur in carnivores held in barren, unchanging enclosures, and may result from inability to obtain sensory information (Clubb and Vickery 2006, Morgan and Tromberg 2007). Aversive stimuli in zoos (e.g. stressful sounds, thwarted escape attempts, housing near natural enemies, incompatible enclosure mates) may also promote stereotypies and induce frustrated escape behavior.

The examples above suggest that locomotor stereotypies may result from frustrated attempts at natural behaviors, such as approaching conspecifics, patrolling an enclosure, or defending a territory (Law et al. 1997, Lyons et al. 1997, Jenny and Schmid 2002, Clubb and Vickery 2006, Quirke and O' Riordan 2011). Overall, it is likely that there are multiple motivations for stereotypies, especially locomotor ones. Clubb and Vickery (2006) pointed out that comparative studies suggest a positive correlation between stereotypic pacing and home range size, while manipulative and observational studies implicate frustrated foraging as the cause and effect factor that instigates stereotypy. Non-motivational factors may also play a role. For example, some stereotypies become habitual and are displayed even without a specific stressor.

Captive felids experience stress in the form of arousal, conflict, and frustration, and manifest that stress through stereotypic behaviors such as excessive grooming and aggressiveness (Law et al. 1997, Lyons et al. 1997, Jenny and Schmid 2002, Krawczel et al. 2005). Such stereotypies can be extremely hard to change in cats exposed to prolonged stress and lack of stimuli (McPhee 2002). Stereotypies involving opioid systems can produce harmful abrasions, alopecia, coat damage, and sores (Willemse et al. 1994, Clubb and Vickery 2006, Farooq et al. 2011). Stereotypies of captive felids involve both behavioral states and events. In animal behavior, an *event* is a behavior that occurs in an instant, while a *state* consists of specific continuous actions (Altmann 1974). For instance, stereotypical pacing is a stress-induced behavioral *event*, whereas inactivity is a common behavioral *state* of captive predators (Quirke and O' Riordan 2011).

Reducing Stereotypic Behavior

Mellen and McPhee (2001) noted that prior to zoos' focus on conservation and captive breeding, stereotypical behavior was not seen as a problem. Once that focus changed, stereotypies were recognized as problematic, but it was not clear how to eliminate them (Mellen and Shepherdson 1997). Subsequently, it was recognized that the frequency of stereotypical states and events can be reduced through properly managed environmental enrichment (Jenny and Schmid 2002, Quirke and O' Riordan 2011). Supplementary tactile, auditory, and olfactory stimuli decrease stress and stereotypical behavior such as pacing, constant grooming, aggressiveness, lethargy, and chewing on cage bars (Lyons et al. 1997, Jenny and Schmid 2002, McPhee, 2002). Because the frequency with which states or events are performed varies with sex, age, and personality (Altmann 1974), certain individuals respond better than others to enrichment measures.

Stress can be reduced in captive felids by exposing them to complex sensory stimuli such as feeding poles, free-swinging feeding sticks, woodpile feeders, and hollow pumpkins containing food (Law et al. 1997, Powell 1997, Jenny and Schmid 2002, Wehnelt et al. 2003). Feeding poles are usually designed for big cats; they consist of a wooden pole with meat loosely suspended high enough for the cat to be required to climb to retrieve the meat. Free-swinging feeding sticks are wooden handles fitted with wooden pegs at one end and hooks at the other; a food item is impaled on the peg and attached by the hook to the roof of the enclosure. Thus the cat does not have easy access to the food item and must put some effort into obtaining it. Woodpile feeders are designed to increase the time and effort used in foraging. Branches piled together form an intricate framework in which food can be hidden (Law et. al. 1997). Partially gutted pumpkins with spices or food inside them also stimulate foraging behavior (Wehnelt et.

al. 2003). Law et al. (1997) and Powell (1997) indicated that combining several forms of stimulation is most effective at decreasing stress and stereotypies, especially for stimuli that mimic animals' experience in the wild (e.g. odors, sounds, and textures associated with hunting and socialization).

Enrichment should take account of species-specific traits and the physical limitations of enclosures. For example, physical stimulation used with a food reward encourages hunting behavior and reduces stress. However, predators lose interest in non-food stimuli if non-food items are used too frequently. Powell's (1997) study on captive ocelots (*Leopardus pardalis*) indicated that the size and structure of exhibits constrain the types of stimuli that can be used. For example, filling water pools more than once a week in exhibits with concrete surfaces may cause ulcers on the foot pads. Powell (1997) also showed that typical species behavior may become less frequent if inappropriate stimuli are provided. In other cases, responses are idiosyncratic. McPhee (2002) described a Siberian tiger (*Panthera tigris altaica*) that seemed to experience extreme stress when a carcass was present in its exhibit.

The Value Of Environmental Enrichment

The phrase "environmental enrichment" is used inconsistently in the literature, but the core concept is improving captive animals' physical and psychological health by creating stimulating environments (e.g. naturalistic enclosures, novel stimuli) (Newberry 1995, Wehnelt et al. 2003, Young 2003, Yu et al. 2009, Quirke and O' Riordan 2011).

Environmental enrichment for zoo felids has become an essential part of their husbandry (Yu et al. 2009, Quirke and O' Riordan 2011). Enrichment of the structure and content of enclosures (e.g. increased size, periodic alteration, natural vegetation, toys) has resulted in increased alertness, higher activity levels, and decreased pacing in a range of felids (Jenny and Schmid

2002, Yu et al. 2009, Quirke and O' Riordan, 2011). Hope and Deem (2006) noted that enrichment also reduces chewing on cage bars. These findings are encouraging because zoo cats often become obese from lack of exercise and experience physical injuries (Law et al. 1997, Hope and Deem 2006). Law et al. (1997) stated that felids in exhibits that lack natural substrates show increased parasite levels. However, when woodchips are used as a substrate and not changed too frequently, parasite levels greatly decrease (Law et al. 1997). African lions (*Panthera leo*) provided with bones in their exhibit showed increased consumptive behavior and decreased stereotypical pacing (Skibiel et al. 2007). McPhee (2002) noted that intact carcasses are often a successful part of environmental enrichment (but see note in previous section regarding one Siberian tiger).

Collateral Benefits and Challenges of Environmental Enrichment Research. Since the 1990s, researchers have identified, characterized, and evaluated different enrichment methods, and discovered several that reduce stress and promote typical species behavior (Powell 1997, Mellen and MacPhee 2001). Other benefits have accrued as well. Observing captive animals in enriched environments is less expensive than observing them in the wild (Law et al. 1997) and provides an opportunity to identify natural behaviors that promote survival (Cagle 2001, Bowman 2004). Law et al. (1997) and Powell (1997) noted that environmental enrichment has provided data on species-typical behavior in feeding and foraging. In addition, enrichment studies have provided insight into poorly understood social behaviors and life history information for species that live in inaccessible habitats (Law et al. 1997, Lyons et al. 1997, Powell 1997, Mellen and MacPhee 2001, Quirke and O' Riordan 2011). Vocalizations authenticated from animals in enriched enclosures may allow researchers to identify species in the field by sound alone. Research on how photoperiod affects breeding allows zoos to coordinate pairings of potential mates to coincide with day lengths conducive to copulation (Law et al. 1997). The practice of coordinated mate-pairing based on photoperiodic response has improved the success of some breeding and reintroduction programs. This is extremely significant because captive populations act as a reserve gene bank for species that are, or may become, extinct in the wild. Environmental enrichment should have a significant role in reintroduction programs because it promotes natural behaviors that are adaptive for animals released into the wild. Enrichment also promotes the educational value of zoo exhibits by displaying natural behaviors, especially those of predatory species, the most popular attractions in zoos (Quirke and O' Riordan 2011).

Despite all this research, there is little direct empirical support in the literature for a connection between for the idea that the enhanced welfare resulting from enrichment actually leads to effective breeding (Carlstead and Shepherdson 1994, Swaisgood 2007). Swaisgood and Shepherdson (2005) argued that problems with data presentation and poor description of enrichment techniques severely limit the conclusions that can be drawn from much zoo research, and that there is little evidence that some enrichments are more efficient than others. There is anecdotal evidence among zoo biologists that enrichment improves reproduction, but little of this derives from controlled experiments reported in peer-reviewed publications. For example, enrichments that promote natural social behavior are widely believed to improve captive breeding success, though few supporting studies have been published. Carlstead and Shepherdson (1994) suggests that captive animals in unenriched environments may be less able to cope with the stress of mating, birthing, and caring for young than are adults with early experience of physically complex settings. Swaisgood (2007) argued that the absence of literature connecting improved welfare with improved reproduction handicaps the zoo community; though it is understood that poor welfare compromises reproductive cycling (Carlstead and Shepherdson 1994, Morgan and Tromberg 2007). The importance of understanding improved welfare is directly united to the importance of discerning what forms of environmental enrichment better promote the goals of enrichment programs.

Olfaction In Mammals

Chemoreception affects behavioral responses in virtually all animals (Stoddart 1980). In vertebrates, the sense of smell achieves great complexity, including diverse receptor molecules and sophisticated neural circuitry in the brain (Doty 1986). Olfactory signals play a significant role in mammalian communication (Doty 1986, Shi and Zhang 2009). When auditory or visual signals are difficult to discern, odors can be used in social situations. Scents provide information about territoriality or space occupancy (Doty 1986). Olfaction is prominently involved in mammalian social life, and the advanced high-level social integration seen in Mammalia might be causally related to olfaction (Stoddart 1980). Qualitative properties, distribution, and intensity of mammalian scent marks provide conspecifics with information about the individuals in an area, such as their reproductive state, physical condition, size, motivational status, stamina, energy level, group constituency, and group size (MacDonald 1985, Doty 1986). The properties of odor signals allow messages to remain in the environment for relatively long periods without endangering the signaling individual, as would visual or auditory cues (Doty 1986). American badgers (*Taxidea taxus*) mostly hunt by olfaction; striped skunks (*Mephitis mephitis*) primarily hunt through olfaction and sound; banded mongooses (*Mungos mungo*) use intrasexual overmarking to promote mating success and discriminate between individuals; brown (*Hyaena brunnea*) and spotted hyenas (*Crocuta crocuta*) use olfaction to locate prey; gray wolves (*Canis lupus*) use olfactory methods in conjunction with cooperative hunting (MacDonald 1985, Conover 2007, Jordan et al. 2011). In Carnivora, species are equipped with numerous odoriferous glands.

Olfaction in Felids. Although felids rely more on visual and auditory cues when foraging, olfaction is consistently used to communicate among conspecifics and investigate novel objects (MacDonald 1985, Macri and Kane-Patterson 2011, Bradshaw et al. 2012). Anal glands, facial glands, urine, and feces are the key sources of chemicals used for olfactory communication in felids (MacDonald 1985, Sunquist and Sunquist 2002), but literature on cat olfactory capabilities appears to be extremely limited. Several studies of enrichment for captive felids focused on olfaction, but the animals' abilities to detect scents were not explored beyond felid behavioral responses (Ellis and Wells 2010, Macri and Kane-Patterson 2011). Odorant receptor (OR) genes are extensively dispersed in the mammalian genome, occurring on nearly all chromosomes with loci differing chiefly in numbers of genes (Fleischer et al. 2009). The size of their olfactory membrane and olfactory bulbs strongly suggest that domestic cats rely heavily on smell (Bradshaw et al 2012). The domestic cat possesses around 200 million scent receptors in the olfactory epithelium, slightly fewer than dogs (*Canis familiaris*), but far greater than humans' roughly 5 million receptors (Case 2003). Sense of smell in felids is also associated with the vomeronasal (or Jacobson's) organ, a structure not found in all mammals (Farbman 1992, Case 2003, Bradshaw et al. 2012). The vomeronasal organ (VMO) is important in the interpretation and reception of pheromonal signals from conspecifics (Holst 1985, Farbman 1992, Sunquist and Sunquist 2002). It is located in the roof of the mouth and consists of paired fluid-filled sacs linked to the mouth and nasal cavities by nasopalatine canals (Case 2003, Bradshaw et al. 2012). The VMO contains receptor cells connected to nerves leading to the amygdala of the hippocampus, a brain region involved with feeding, sexual, and social behaviors in felids (Holst, 1985; Case, 2003). Passive flow of odor molecules to chemoreceptors in the VMO is doubtful, whereas every time a cat breathes scent molecules are received by the olfactory epithelium passively (Bradshaw et al. 2012). The flehmen response is an external sign that the VMO is being used as animals inhale odors into the nasopalatine canals (Case 2003, Bradshaw et al. 2012). Three families of receptor proteins occur in the VMO: V1Rs, V2Rs, and FPRs (Bradshaw et al. 2012). In felids only the V1Rs have been studied and they vary among species. Dogs have no functioning V2Rs, and felids may be similar (Bradshaw et al. 2012). Differences in sensory ability among felid species are largely unknown due to lack of research; it is possible that all felid species possess the same olfactory structure and scent receptors as the domestic cat, but there are no data to support this assumption (Bradshaw et al. 2012). Fraser (2012) suggested that big cats could have more or fewer scent receptors than smaller species, and those olfactory abilities could be related to hunting styles. Senses of smaller cat species are adapted to close-up inspection, in contrast to larger species whose senses are developed for long range identification of prey. Big cats have smaller eyes relative to the area of the rostrum than do smaller cats. These physiological and anatomical differences could indicate that larger felids have stronger olfactory capabilities than their smaller cousins (Fraser 2012, Montague et al. 2014).

A stereotypical felid response to olfactory stimulation is the sniff (Case 2003). Sniffing involves a series of short and rapid inhalations and exhalations that allow odorant molecules to remain in the nasal passage for prolonged periods due to disruption of normal breathing (Case 2003). The duration of scent molecules in the nasal passage could also vary by species, so it cannot be assumed all cat species achieve the same olfactory sensitivity by sniffing. Olfactory behaviors in felids vary by sex (MacDonald 1985). For example, male lions and both sexes of tiger (*Panthera tigris*) spray more often than female lions. Cheetahs and other felid species likewise have different spray marking behaviors than lions.

Olfactory Enrichment. Despite the growing body of research on environmental enrichment for felids, few studies have investigated the effect of olfactory enrichment (Law et al. 1997, Wells and Egli 2004, Thomas et al. 2005, Quirke and O' Riordan 2011). Clark and King (2008) noted that different felid species react differently to olfactory stimuli, but justifications for using particular scents as enrichments are almost absent in the literature.

Hill et al. (1976) completed a study at the Knoxville Zoological Park that examined felid reactions to catnip, *Nepeta cataria* (Labiatae). The study involved six species: African leopard (*Panthera pardus*), African lion, bobcat (*Lynx rufus*), jaguar (*Panthera onca*); mountain lion (*Puma concolor*), and tiger. The authors concluded that jaguars and African lions responded to catnip, but bobcats, mountain lions, and tigers did not. Reproductive adults were more responsive to the enrichments than immature and post-reproductive individuals. This was among the first reports to indicate that big cats in zoos respond to introduced olfactory stimuli and that the response is species specific.

Following the observation by Powell (1995) that scents could function as environmental enrichments for captive African lions (*Panthera leo*), Yu et al. (2009) observed the responses of six Amur leopards (*Panthera pardus orientalis*) in the Beijing Zoological Garden to different olfactory stimuli. These animals had displayed stereotypic behavior and excessive inactivity, and the focus of the research was to determine whether olfactory stimuli would increase their behavioral diversity, physical fitness, and psychological health. The study measured responses to nutmeg (*Myristica fragrans*), feces of roe deer (*Capreolus capreolus*), and urine from Siberian tigers representing herb, prey, and predator scents, respectively. Exposure to all scents increased behavioral diversity significantly, with nutmeg producing the longest duration of response (3 hours). During the nutmeg enrichment, most new behaviors observed were classified as playful; in contrast, investigative behaviors were common when tiger urine and roe deer feces were introduced. The enrichments also increased the leopards' spatial use of their exhibit areas. Thus Yu et al. (2009) established that olfactory stimuli can achieve the purposes of enrichment by encouraging species-specific behaviors and reducing stereotypies in Amur leopards.

Skibiel et al. (2007) demonstrated that olfactory stimuli could serve as environmental enrichments for captive African cheetahs. This study measured the responses of six felid species, including African cheetahs, to three spices (chili powder, cinnamon, and cumin) introduced into exhibits at the Montgomery (Alabama) Zoo. Activity levels of the cheetahs increased during the olfactory treatment period. Quirke and O'Riordan (2011) reported on the effects of scimitar-horned oryx (*Oryx dammah*) feces as an olfactory stimulus for 12 adult cheetahs. During exposure to the scent, cheetahs had prolonged and positive behavioral responses, increased explorative behavior and locomotion, and decreased vigilance and pacing. Post-treatment, locomotion increased and inactivity decreased (Quirke and O'Riordan 2011). Like Skibiel et al. (2007), this study revealed that olfactory stimuli can be successful enrichments for African cheetahs, encouraging species specific behavior while reducing stereotypies.

The foregoing studies have established that olfactory stimuli may serve as enrichments for captive African cheetahs (Skibiel et al. 2007, Quirke and O'Riordan 2011). These experiments used natural scent types (spices and feces of other species) for enrichment, but Thomas et al. (2005) utilized commercial fragrances to attract wild African cheetahs to hair sampling sites and as olfactory enrichment for two captive African cheetahs in Bronx Zoo. Thomas et al. (2005) focus was to determine if the captive cheetahs would respond to the fragrances with cheek rubbing behavior. Anecdotal evidence from zoo keepers suggested that commercial fragrances may provide effective olfactory enrichment, and only the Thomas et al. (2005) study has used such fragrances to encourage species specific behaviors of African cheetahs. All commercial fragrances are derived, to a greater or less extent, from scents in nature (Sell et al. 2006). Because olfaction is used in foraging and communication (Doty 1986; Sell et al. 2006), there could be considerable value in identifying readily available odorants for captive enrichment.

The purpose of this study was to compare behavioral responses of captive African cheetahs to six types of commercial fragrances used as olfactory enrichment stimuli. Three of these fragrances are marketed as “men’s cologne” and three as “women’s perfume”. The products mimic natural scents described as “musk”, “spice”, and “floral”. Each of the products was categorized by a fragrance specialist. My first objective was to determine whether African cheetahs respond to any of these scents with a variety of natural engagement behaviors. My second objective was to assess whether the cats display preferences for specific scent categories (i.e. men’s vs. women’s, musk vs. spice vs. floral).

CHAPTER 2

METHODS

Procedure

Sample Size of Cheetahs. I worked with four adult cheetahs in the St. Louis Zoo, three males and one female. Cheetahs were observed in their zoo enclosure for 7-15 weeks. The sample size was small, but similar studies have been completed with two (Powell 1997, Jenny and Schmid 2002), four (Krawczel et al. 2005), six (Wooster 1997, Yu et al. 2009), nine (McPhee 2002), 12 (Quirke and O' Riordan 2011), 14 (Skibiel et al. 2007), or 19 individuals (Lyons et al. 1997). Cheetahs are not a common species in zoos and therefore the number of individuals available is limited.

Olfactory Stimuli. The focus of this study is olfactory enrichment in the form of commercial fragrances (i.e. colognes and perfumes). Fragrances were purchased from Macy's in Carbondale, Illinois in September 2012, and classified with the help of a fragrance specialist as musky, spice, and floral based on the mixture of natural and artificial chemicals of which they are composed. Fragrances were also classified by target gender, male (cologne) and female (perfume). I included three male and three female fragrances that included examples of all three fragrance types. The specific commercial products used are shown in Table 1 and hereafter referred to by two letter acronyms (e.g. MM = Male Musky = Ralph Lauren Double Black®).

Each fragrance container was opened with a different tool to prevent chemical carryover from the other fragrances. The fragrances were poured into individual 100ml beakers. A 100ml pipet was used to transfer 1ml aliquots of each fragrance to individual cryogenic tubes. The tubes were marked with codes to identify the fragrances. Fragrance tubes were placed in separate containment boxes to keep all fragrances separate and organized (Figure 1). The fragrances were stored at < 0° C so their chemical compositions would not change over the course of the study.

Experimental Design

Observations began in October 2012 and ran through December 2012, and were conducted on days with temperatures above 4.44°C and little to no precipitation. Bouts were continued the next year starting in May 2013 and ending in April 2014. The cheetah enclosure at the St. Louis Zoo is approximately 1.6 ha, with eight main pens and two smaller feed yards. These areas are connected through a central building, and access is regulated by gates and guillotine doors (Figure 2).

Scent dispensers were constructed to allow cheetahs to interact with the enrichment chemical, but prevent them from ingesting it (Figure 3). The scent dispensers were PVC pipes 30.48cm in length with small holes drilled into them; one end of the pipe was enclosed by a glued slip cap. The other end had a special female adapter and a CPVC male adapter plug, allowing a fragrance to be inserted and removed easily. Two screw eye hooks were placed in each scent dispenser for the attachment of a chain. Chains were chosen to hold the dispensers because materials like rope can easily be chewed. Chains were cut to wrap around specific trees in each cheetah enclosure (Figure 4). Clips held the chains to the scent dispensers, and colored plastic twist-ties were attached to the chains for identification. Twelve scent dispensers were made for the study, six (one per scent) for the female cheetah and six for the males. The female cheetah received her own set of dispensers to prevent transmitting her scent into the males' enclosures, or vice versa. To prevent the mixing of different fragrances, a code was written on each scent dispenser. The codes identified which dispensers were strictly for males and the female (Figure 3). A plastic bag was assigned to each dispenser and the same code written on the bags to prevent fragrances from carrying over to other dispensers. Scent dispensers designated for males were placed in a separate larger bag from those intended for the female.

All cheetahs were given empty scent dispensers in their exhibit on two separate days to remove the effect of novelty behavior, i.e., behavior instigated purely by curiosity over a new item in the enclosure. Observations were scheduled so that each male fragrance was compared to each female fragrance in a single observational bout that involved one animal (cheetahs did not share enclosures) with both scents placed in the enclosures simultaneously at various distances depending on the enclosure. The trees used in the experiment were alternated so the same scent dispenser was not placed at the same tree the next day to prevent scent carryover. Trees that could be seen easily by observers and were on the pathway the cheetahs periodically patrolled were chosen as the scent locations (Figure 2). Fragrances were thawed before the start of each bout. Surgical gloves were worn to prevent observer scent from transferring with the fragrances. A 1ml aliquot of fragrance was placed on a clean gauze pad in the middle of each of the two scent dispensers, which were placed in the enclosure by a carnivore keeper. New gloves were worn for each fragrance tube in case fragrances squirted out in the process of applying them to the gauze pads.

An observational bout began with a cheetah that was awake or patrolling its exhibit. Once the keeper left the enclosure and locked the gates, the study bout time was recorded with a stopwatch. Each observation bout was 30 minutes. Once a bout was completed scent dispensers were cleaned and the gauzes removed. The scent dispensers were air dried and placed in their individual plastic bags to prevent scent carryover.

Behavioral responses to enrichment stimuli were assessed in a variety of ways. Previous studies used ad libitum behavior sampling prior to project observations to help develop an ethogram, with observational periods scheduled near peak activity times (Skibiel et. al 2007, Yu et al. 2009) An ethogram developed by Mossotti (2010) for cheetah behavior was used in this study (Table 2). All behaviors described in Table 2 are common in wild cheetahs, but may be rarer in captive ones, and were considered as positive engagement responses to olfactory stimuli (except hissing, an aggressive response). I quantified responses by their durations, and recorded the dispenser (scent) toward which the cheetah was displaying interactive behavior. There were nine combinations of female and male fragrance comparisons repeated four times for a total of 36 bouts, equal to 1080 minutes of observation for each cat (Table 3). Bouts were cancelled (and later repeated) if an individual was asleep or sick during the entire bout, or if weather conditions prevented observations. The bouts were scheduled after feedings to prevent cheetahs from associating dispensers with food. During each set of bouts all four cats were observed on the same day in random order to be consistent with circadian rhythms. Observations were recorded on a cheetah behavior data sheet (Figure 5).

Data Analysis

Due to the small number of subjects, this study may lack statistical power and will only be able to detect strong odor preferences. In the context of this exploratory research, I tested two null hypothesizes. Null hypothesis 1: There was no difference in the amount of time cheetahs spent interacting with male versus female fragrances. Null hypothesis 2: There was no difference in the amount of time cheetahs spent interacting with the three fragrance types.

I used repeated measures ANOVA (SPSS software) with interaction time as the response (dependent) variable. My observations rely on a small sample size and reflect individual personalities of cheetahs, so repeated measures ANOVA is an appropriate test because the same individuals are being measured repeatedly at all levels (Girden 1992, Laerd Statistics 2013).

ANOVA requires one or more independent variables and one dependent variable, and is used to assess the significance of differences in mean scores of the dependent variable under three or more conditions. The independent variable has categories called levels or related groups. Table 3 illustrates the study design, with four cheetahs as subjects (A to D) interacting with nine different conditions (T1 to T9). The independent variables were the nine conditions tested, and the dependent variable was interaction time. A repeated measures ANOVA removes variability due to differences between subjects from the total within-groups variability, resulting in a smaller error variability (SS_{error}) and increasing the value of the F-statistic. Test power is increased when the repeated measurements are treated as independent to discover differences between means. Values of $SS_{between,}$ $SS_{within,}$ $SS_{subjects,}$ and SS_{error} were calculated to obtain $MS_{between}$, and MS_{error}, and $F = \frac{MS_{between}}{MS_{error}}$. $MS_{between}$ was calculated as $MS_{between} = \frac{SS_{between}}{df_{\text{between}}}$, and MS_{error} was calculated as $MS_{error} = \frac{SS_{error}}{df_{\text{error}}}$. The $df_{between}$ was calculated as $df_{between} = a - 1$; where a refers to the number of levels. df_{subjects} was calculated as $df_{subjects} = s - 1$, the letter s indicates the number of subjects. df_{within} was calculated as $df_{within} = N - a$ with N referring to the total number of measurements taken, and df_{error} was calculated as $df_{error} = df_{within} - df_{subjects}$. The significance level was $\alpha = 0.05$. If the F-statistic is greater than the critical value in the F table for the appropriate degrees of freedom then the null hypothesis is rejected. Data obtained from the observational bouts had a non-normal distribution and so were normalized by logarithmic transformation. I also tested the null hypothesis of no difference in interaction times between with loaded versus empty dispensers to determine whether the cheetahs were more interested in scents than scent dispensers. A paired t-test was used and the data were normalized by logarithmic (base 10) transformation.

CHAPTER 3

RESULTS

Total interaction times of each cat with each scent are shown in Table 4 (raw data from observational bouts are in Appendix A). All behaviors in Table 2 with the exceptions of flehmen and hissing occurred during at least some of the bouts. The male Kgosi was excluded because he did not interact with scents in any of the bouts. During the 1080 minutes of observation, Jason spent 4.84%, Zuri 0.85%, and Shanto 0.41% of the time interacting with the scent dispensers (Figures 6-8). Jason displayed seven different behaviors, Zuri displayed five, and Shanto displayed four. The dominant behavior with all cheetahs was proximity. Jason's and Shanto's second most common response was to sniff, and Zuri's was to paw at the dispensers.

There was no significant difference in the amount of time cheetahs spent with the dispensers during novelty days and scent trials (Table 5).

Cheetahs spent more time interacting with male than with female fragrances in total (Tables 4, 6, 7); this was also true for each individual (Figures 9-11). Interaction times with male fragrances showed less variation than those with female fragrances (Figure 9). The outlier in Figure 9 represents a data point that is greater than 1.5 interquartile ranges away from the 75th or 25th percentiles and was therefore excluded from descriptive statistics and analyses.

Medians and means for interaction times with scent types were in the rank order spice > musky > floral (Table 8 and Figure 13), but I found no significant difference in the amount of time spent interacting with the three fragrance types (Table 9). The boxplot in Figure 12 indicates that variation in total interaction time spent with musky and spice scents, but cheetahs were more consistent in interaction times with floral scent. The outlier aforementioned represents an observation that was significantly higher than the other expected scores (Figure

12). Individual cheetahs spent similar amounts of time interacting with floral fragrances; two spent slightly more time interacting with spice and musky scents, but the third showed no preference (Figures 13-14).

CHAPTER 4

DISCUSSION

My first objective was to determine whether African cheetahs responded to any of the commercial scents with natural engagement behaviors. The second objective was to evaluate whether the cats displayed significant preferences for specific scents (i.e. men’s vs. women’s, musk vs. spice vs. floral). I found that cheetahs responded to the scent enrichments with documented engagement behaviors (Figures 6-8). They appeared to prefer men’s over women’s fragrances, but had no significant preference among musk, spice, and floral types (Tables 6 and 9). There was no significant difference during novelty days and scent trials (Table 5). However, the low sample size reduced the statistical power to detect any but a very large significant difference (Northwestern University 1997, Steidl et al. 1997). These results raise several questions for further analysis and may have implications for the use of fragrances as effective enrichments.

The few previous studies of olfactory enrichment in felids also documented engagement behavior with introduced scents (Ellis and Wells 2010, Macri and Kane-Patterson 2011). Thomas et al. (2005) used fragrances in attempt to attract wild African cheetahs to hair sampling sites, and to elicit cheek rubbing behavior in two captive African cheetahs in the Bronx Zoo. Behaviors varied among the three individuals in my study, but both males sniffed the fragrances more often than did the female (Figures 6-8). Female African cheetahs can use olfaction to discriminate among males when choosing a mate (Mossotti 2010). Case (2003) noted that a domestic cat apparently can discriminate among urine scents of individuals from its own group, neighboring groups, and strangers. Thus, several indirect lines of evidence suggest that cheetahs have sufficient olfactory sensitivity not only to detect the scents used in this study, but be aware that the scents were not from another cat.

Chemistry Of Fragrances

All six fragrances I used include essential oils, which are volatile and aromatic liquids that determine the characteristic odor of a plant. Each is extracted by distillation from a single plant species (Lawless 1995). Modern commercial fragrances include both natural and synthetic components (Turin and Sanchez 2008), the latter providing additional scents and non-fragrant properties (such as sun screening and waterproofing). Products used in this study were categorized as spice, musky, and floral by the fragrance industry on the basis of three “notes”. Notes describe the oil composition and evaporation rates of a commercial fragrance. Top/head notes usually evaporate within one hour and constitute 10-30% of the amalgam: middle/heart notes last several hours and are 30-60% of amalgams, base/bottom notes can last a day or more and are 15-30% of amalgams. I was unable to determine the exact percentages of each chemical component due to the proprietary information policies of the fragrance companies.

Spice Scent Types. The chemical components of Calvin Klein’s Women’s Obsession® and Men’s Obsession® are shown in Tables 10-11. Cinnamon (obtained from the bark of *Cinnamomum verum*) and its components such as cinnamon oil were main ingredients in both fragrances. Skibiel et al. (2007) demonstrated that spices including cinnamon can serve as environmental enrichments for captive African cheetahs. My findings are consistent with this observation.

Musky Scent Types. The two musk types used in the study, Ralph Lauren Double Black® for men and Yves Saint Laurent Opium® for women attracted the attention of three of the cheetahs. Both fragrances were composed of numerous natural and synthetic ingredients (Tables

12-13). Opium has several ingredients deriving from cinnamon while Double Black has some ingredients found in lemongrass. Both natural ingredients have been successfully used as olfactory enrichment for felids (Wehnelt et al. 2003, Skibiel et al. 2007).

Floral Scent Types. Paco Rabanne's 1 Million® and Marc Jacobs Daisy® chemical compositions are included in Tables 14-15. Both fragrances contained natural chemicals found in catnip, lemongrass, and cinnamon. The floral fragrances also contained butylphenyl methylpropional, a synthetic floral odor not found in any other fragrances used in this study.

Common Chemical Differences. Inspection of Tables 4 and 6 shows the significant preference for men's fragrance was dominated by male cheetah Jason's interest in the male musky scent Double Black. Comparison of the ingredients in Tables 10-15 reveals that Double Black has seven volatile chemicals that occur in few of the female fragrances tested: hexyl cinnamal, amyl cinnamal, isoeugenol, citronellol, citral, unknown D29099/1, and unknown 645335. Nothing can be said about the unknowns, but there is a large literature on the other chemicals that includes some potential clues for understanding the cheetahs' interest. For example, Vaglio et al. (2009) presented experimental evidence that hexyl cinnamal is a human pheromone that allows infants to recognize their mothers. The lemon scent citral functions as an alarm pheromone in acarid mites (Kuwahara and Suzuki 1983) and as a male sex pheromone in green-winged butterflies (Pieris: Lepidoptera, Andersson et al. 2007); the sequence of its odorant receptor protein is relatively conserved in tortricid moths (Carraher et al. 2012). However, lack of flehmen behavior during observational bouts suggests that cheetahs were not detecting these chemicals via vomeronasal pheromone receptors. Citronellol is a major component in volatile oil from the catnip herb (Baranauskiene et al. 2003) and lemongrass (*Cymbopogan citratus,* Poaceae), both of which have been documented as effective olfactory enrichments for felids (Hill et. al. 1976, Wehnelt et al. 2003, Wells and Egli 2004, Ellis and Wells 2010, Resende et al. 2011, Piaru et al. 2012). The citronellol olfactory receptor has been identified in pigs (*Sus scrofa*; Silva et al. 2014) and humans (Schmiedeberg et al. 2007). Fur seals (*Arctocephalus pusillus*) can discriminate between D and L enantiomers of citronellol (Kim et al. 2013), although primates cannot (Laska et al. 1999). Citronellol has hypotensive and vasorelaxant effects in rats (Bastos et al. 2009), and reduces orofacial pain-response behavior in mice (Brito et al. 2013). It is an aggregation pheromone in ambrosia beetles (*Platypus spp.*; Kim et al. 2009). Taken together, these studies suggest that several chemicals in the Double Black fragrance have complex and diverse signaling functions in animals. None have been tested specifically in cheetahs, but my results suggest that research along these lines might be fruitful.

Variables Affecting Experimental Results

Sample Size Effects. The study relied on only four cheetahs, making generalization of my results questionable and severely limiting the power of my statistical tests. The small number of cats resulted in difficulty obtaining data when an individual was sick or uninterested during an observation bout. Bouts were rescheduled in these situations, reducing the total number of informative bouts, given limits on the duration of the study. Observing so few individuals may also have biased the observed frequencies of exploratory behaviors in Table 2. Idiosyncrasies of individual cheetahs affect their behavioral responses (Appendix B), and a larger sample of cats is the only way to minimize this bias. Jason demonstrated a much stronger interest than the other cats in the men's musky fragrance Double Black. Jason also displayed seven behavioral responses to the scent dispensers compared to Zuri's five and Shanto's four (Figures 6-8). Despite Zuri and Shanto showing a slight bias towards men's fragrances, Jason's responses dominated the study, and this complicates interpretation of the final results.

Bout Time Limitations. Observation bouts lasted 30 minutes and this duration was chosen somewhat arbitrarily. Longer bouts might have accommodated the cats' personalities and minimized the influence of distractions. The typical interval between a cat's first exposure to a fragrance and detection of the fragrance, or between detection and behavioral engagement, is unknown. Perhaps the cheetahs would have displayed more engagement if I watched them longer. During some 30-minute intervals, other interests may have taken immediate priority over enrichments, though the cats might choose to interact with the enrichments later. Alternatively, a cheetah's interest in novel scents can decline very quickly after detection, such that shorter observation bouts would make immediate responses more noticeable. The cheetahs' behavioral interactions with the scent dispensers varied considerably within single bouts. Clearly, exploring the affect of bout duration on behavioral responses would be appropriate.

External and Internal Distractions. Sensory distractions were common during the study bouts. A few times, food remained in the enclosure and was preferred over interacting with fragrances, though the cheetahs later displayed interest in the enrichments. During the first two weeks, three cheetahs showed interest in me and my assistant during the bouts. These cats were not accustomed to being observed constantly; for example, Jason took time to watch me and my assistant while interacting with the fragrances. There was no indication of discomfort, however, and the cats often purred while observing us. The St. Louis Zoo has a train that passed near the cheetah enclosures during bouts. All four cheetahs paid attention to the train, and its horn was a cue alerting them it was passing. Hearing and seeing the people on the train was a strong enough stimulus for the cats to stop sleeping or resting, and change patrol patterns to obtain a clear view of the train as it passed. On the other hand, people walking by the main exhibit housing Shanto seemed not to distract him.

Native wildlife able to enter the exhibits did not catch the cheetahs' attention very often during bouts, possibly because the cats had already been fed and were accustomed to wildlife. Gray squirrels (*Sciurus carolinensis*) attracted the most attention from all cheetahs. Asian elephants (*Elephas maximus*) were a common distraction for Jason and Zuri because the elephants were visible from their enclosures. In some bouts, Zuri spent the most time watching the elephants. Feces from the elephants also drew attention away from fragrance dispensers and initiated a flehmen response from Zuri, a behavior never displayed towards the enrichment scents. During one bout camel hair was also present in the exhibit and attracted the cats' attention.

Communication between cats was very brief when it did occur. Most such communication was between the siblings Zuri and Kgosi. Chirping and smelling each other were their main forms of communication. In October 2013, Jason was paired with a female cheetah not involved in the study a day before an observational bout. The female had gone into estrus earlier that week, and Jason still demonstrated interest in her during the bout even though he was placed back into his enclosure alone.

Weather Conditions. Cheetah responses were heavily dependent on weather conditions. In most cases, activity levels were high when temperatures were 19.4-25°C (Figure 15). Cheetahs ignored the scent dispensers when temperatures increased above this range. There was a complete behavioral change in all four individuals when temperatures reached 30°C or higher during the day. Patrolling behavior stopped and all four individuals moved into shaded areas. Heavy rain and thunderstorms forced a few bouts to be rescheduled. Illness (accompanied by nasal congestion and sneezing) forced some bouts to be deferred until the cheetahs were completely healthy.

Clearly, the cheetahs' responses to scent dispensers were influenced by ambient temperature of cheetah thermoregulation effected by temperature. Scent molecules break down at different rates, depending on environmental and atmospheric conditions, which affect a predator's capability to detect them (Conover 2007). Certainly the vapor pressures of compounds in the fragrance mixtures increase with temperature, such that volatiles are diffused more quickly in warmer conditions. Kuehn et al. (2008) noted that in humans the ability to detect odors increases with increased temperature and humidity. Higher temperature and humidity may increase the capacity of air to transport odorant molecules, with more of them being bound to water vapor (Kuehn et al. 2008). Cheetahs spent time on all enrichments when temperatures were warm, but seemed to prefer hay over anything else when temperatures were 13°C or colder.

Improving The Experimental Design

This research led to interesting results and new questions about African cheetah enrichment. However, several aspects of the experimental design should be changed if the experiment were to be repeated. These improvements would lead to greater statistical power and reduced bias.

Aspects of Bout Design.

It would be beneficial for more data to be collected on cheetah behavioral responses with empty scent dispensers. This would allow assessment of cheetahs' response to the novelty of observation bouts, and also provide enough data to test whether cheetahs spent more time engaging with loaded scent dispensers than with empty ones.

Increasing the bout time from 30 to 60 minutes might be more accommodating to the cheetahs' personalities and minimize the influence of distractions. More time might also give observers a greater chance of seeing exploratory behaviors when the cats interact with fragrances. The bouts were scheduled to occur in consecutive weeks, but the most consecutive weeks completed were four. The other weeks were interrupted for reasons previously mentioned. Scheduling bouts at multiple intervals would be a better design, allowing a test of whether a too frequent enrichment becomes uninteresting to the cats. Bassett and Buchanan-Smith (2007) stated that predictability of an event is known to affect an animal's reaction to it.

My data suggest that a boredom effect occurred for three to four weeks in August 2013. During this time, there was a clear behavioral change with all three cats. Although they watched the keeper set up the dispensers, they showed no interest in the dispensers afterwards. During this period almost no data were collected. After a delay of >1week, bouts were continued in September 2013; Jason and Zuri immediately displayed behavioral responses at rates similar to those previously recorded, and Shanto began responding likewise a week later.

Increasing the Sample Size. A larger sample of cats would give greater statistical power and reduce the effect of individual personalities on the results. In addition, factors such as age, sex, and prior enrichment exposure could be included in the experimental design. As noted in the Introduction, this will require extensive travel to multiple institutions.

Prospects For Olfactory Enrichment With Commercial Fragrances

Based on the results of this research, commercial fragrances may be a useful olfactory enrichment option for African cheetahs. I suggest that commercial fragrances be used as seasonal enrichments due to limitations related to weather (e.g. freezing temperatures, severe thunderstorms). The fragrances will freeze in cold temperatures, eliminating their effectiveness as olfactory stimuli. They should be used sporadically so cats are unable to predict enrichment presentation patterns. Similarly, fragrances could be sprayed in different areas of a cat's exhibit at different times to reduce spatial predictability. Fragrances could also be combined with other enrichments (e.g. chew toys, scratching logs), and specific fragrances given to specific cats based on their individual responses. In these respects, commercial fragrances present more diverse options than some other enrichment stimuli.

Commercial fragrances do require special care. They must be frozen to prevent chemical breakdown and kept in separate containers to maintain their distinctive qualities. On the other hand, they are relatively inexpensive enrichments, given that most are sold in volumes containing over a year's supply of product.

The commercial fragrances I used are composed of the chemicals listed in Tables 10-15. Some of the natural products in the fragrances have already been used successfully as olfactory enrichments for felids. Other natural components and commercial fragrances could be tested to determine their value as olfactory enrichment. This information could be used to develop a scheduled enrichment plan in which fragrances are used seasonally, while other volatile chemicals (e. g. cinnamon) are used year-round.

CHAPTER 5

CONCLUSION

The focus of this study was to assess the behavioral responses of four adult African cheetahs to commercial fragrances as olfactory enrichments. The results suggest a significant difference in interaction times with male and female scents; the cheetahs interacted more with male fragrances. There were no significant differences in interaction times with musky, spice, and floral fragrances. These conclusions are tentative in light of the study’s small sample size, but suggest that use of commercial fragrances as olfactory stimuli for African cheetahs merits further investigation.

Table 1. *Fragrances used in this study.*

Fragrance Type	Men	Women
Musky	Ralph Lauren Double Black (MM) Macy's Product # 222224	Yves Saint Laurent Opium (WM) Macy's Product # 458913
Spice	Calvin Klein Men's Obsession (MS) Macy's Product # 465460	Calvin Klein Women's Obsession (WS) Macy's Product # 5464
Floral	Paco Rabanne One Million (MF) Macy's Product # 591194	Marc Jacobs Daisy (WF) Macy's Product # 276420

Table 2. *Cheetah ethogram from Mossotti (2010).*

Behavior	Description
Proximity (PR)	The animal comes within one body length of the scent dispenser but does not touch it or perform any other behaviors toward it. The behavior ends when the animal moves at least one body length away from the scent dispenser. **State: Record start time and end time.**
Sniff (S)	The animal brings its nose to or within one head length of the scent dispenser. **State: Record start time and end time.**
Lick (L)	The animal makes contact with the scent dispenser with its tongue.
Bite (B)	The animal places its mouth around the scent dispenser.
Paw (P)	The animal makes contact, using one or both front paws, with the scent dispenser.
Flehmen (F)	The animal raises its head to the horizontal plane and grimaces after smelling or licking the scent dispenser.
Scent Roll (SR)	The animal simultaneously rolls and rubs its entire body on the ground, on the scent dispenser or within one body length.
Urinate (U)	The animal releases urine within one body length of the scent dispenser.
Hiss (H)	The animal brings its head to or within one head length of the scent dispenser, opens mouth and forcibly expels air towards the scent dispenser.

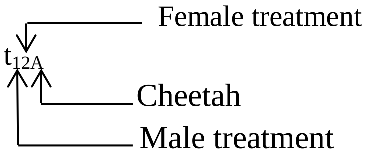
Table 3. *Physical description of treatment testing, each treatment was repeated four times totaling 36 bouts.*

African Cheetah Olfactory Enrichment Study

Subjects	**Treatments**								
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
A	t_{11A}	t_{12A}	t_{13A}	t_{21A}	t_{22A}	t_{23A}	t_{31A}	t_{32A}	t_{33A}
B	t_{11B}	t_{12B}	t_{13B}	t_{21B}	t_{22B}	t_{23B}	t_{31B}	t_{32B}	t_{33B}
C	t_{11C}	t_{12C}	t_{13C}	t_{21C}	t_{22C}	t_{23C}	t_{31C}	t_{32C}	t_{33C}
D	t_{11D}	t_{12D}	t_{13D}	t_{21D}	t_{22D}	t_{23D}	t_{31D}	t_{32D}	t_{33D}

T1 = Male musky scent vs. Female musky scent
T2 = Male musky scent vs. Female spice scent
T3 = Male musky scent vs. Female floral scent
T4 = Male spice scent vs. Female spice scent
T5 = Male spice scent vs. Female musky scent
T6 = Male spice scent vs. Female floral scent
T7 = Male floral scent vs. Female spice scent
T8 = Male floral scent vs. Female musky scent
T9 = Male floral scent vs. Female floral scent

t = Total time summed over bouts



A = Shanto
B = Zuri
C = Jason
D = Kgosi

Table 4. *Total amount of time (seconds) cheetahs interacted with all scents.*

Cheetah	**MS**	**WS**	**MM**	**WM**	**MF**	**WF**
Shanto	13	42	43	8	82	15
Jason	334	360	2236	109	26	70
Kgosi	0	0	0	0	0	0
Zuri	182	53	41	130	138	12
Totals	498	453	2303	240	247	75

Table 5. *Paired sample t-test for scent dispensers with no scent vs. scent dispensers with scents based on log_{10}-transformed data with 95% CI. The scent dispensers with no scent were given for two days, and then scent trial days began. The p-value indicates no significant difference among the dispensers.*

Mean	Standard Deviation	Std. Error Mean	t	df	P-value
-2.895	2.127	1.228	-2.358	2	0.142

Table 6. *Descriptive statistics for interaction times of cheetahs with male and female fragrances excluding the outlier. The units are log_{10}-transformed seconds.*

	Male	Female
Mean	1.99	1.65
Median	1.91	1.68
Std. Deviation	.666	.536
Range	2.24	1.65
Minimum.	1.11	.90
Maximum	3.35	2.56
CIs	95%	95%

Table 7. *Repeated measures ANOVA results for male vs. female fragrances based on log_{10}-transformed data in Table 4. The row named male vs. female indicates the significant difference in total time interaction and the row named cats indicates significant difference among the cheetahs.*

	Sum of Squares	Df	Mean Square	F	P-Value
Male vs. Female	1.329	1	1.329	652.928	.001
Cats	6.716	2	3.358	1648.973	.000
Error(male vs. female)	0.004	2	0.002036		
Total	8.050	5			

Table 8. *Descriptive Statistics of Scent Types Compared excluding the outlier. The units are* $\log_{10}$*-transformed seconds.*

	Spice	Musky	Floral
Mean	1.95	1.94	1.56
Median	1.99	1.83	1.54
Std. Deviation	.566	.812	.418
Range	1.44	2.45	1.06
Minimum	1.11	.90	1.08
Maximum	2.56	3.35	2.14
CIs	95%	95%	95%

Table 9. *Repeated measures ANOVA results comparing scent types based on* $\log_{10}$*-transformed data in Table 4. The row named scents indicates the no significant difference in the amount of time spent interacting with the three fragrance types and the row named cats indicates no significant difference.*

	Sum of Squares	Df	Mean Square	F	P-Value
Scents	1.045	2	0.522	0.846	0.493
Cats	4.477	2	2.238	3.623	0.126
Error(scents)	2.471	4	0.617		
Total	7.994	8			

Table 10. *Chemical composition of women's Calvin Klein Obsession (listed by Calvin Klein®).*

Chemical Name	Chemical Composition
Natural Components	
Cinnamal (3-phenyl-2-propenal)	The main component of cinnamon oil (Ackermann et al. 2009)
Cinnamyl alcohol	A component found in cinnamon oil (Ackermann et al. 2009)
Citral	An acyclic α, β-unsaturated monoterpene aldehyde that naturally occurs in the essential oils of the May Chang (*Litsea cubeba*) also known as the mountain pepper. Citral is also an isoprenoid compound made up of the isomers geranial and neral (Yu Li et al. 2014)
Citronellol	Industrially it is an important essential oil found in catnip and lemon grass (*Cymbopogan citratus*) (Resende et al. 2011; Piaru et al. 2012; Kakaraparthi et al. 2014)
Coumarin	Found in the tonka bean, it is a fermented substance that has a vanilla identical aroma and is used as a vanilla substitute (Columbia Encyclopedia 2013).
eugenol	Found in cinnamon oil one of its many components (Ackermann et al. 2009)
Farnesol	An acyclic sesquiterpene frequently used to emphasize the odors of scents and is widely distributed in many essential oils such as citronella, neroli, cyclamen, lemon grass, tuberose, rose, musk, and balsam (Ham 2013)
Geraniol	Found naturally in lemongrass (Fisher and Phillips 2008)
Limonene	Found in citrus oils and considered is volatile in nature (Fisher and Phillips 2008)
Linalool	Found in various citrus oils and is the main component extracted from the orange blossom (Fisher and Phillips 2008)
Aqua/water/eau	Nontoxic water
Synthetic Components	
Amyl cinnamal	A mostly synthetic a pale yellow liquid with a floral smell reminiscent of jasmine (Cosmetics Info 2016c)
Benzyl cinnamate	A fragrance element used in numerous fragrance compounds (Bhatia et al. 2007)
Benzyl alcohol	A primary alcohol and is a member of the fragrance structural group Aryl Alkyl Alcohols (Scognamiglio et al. 2012)
Hydrolyzed jojoba esters	Derived from a reaction of oil from the jojoba plant (*Simmondsia chinensis*) and potassium hydroxide (Meyer et al. 2008)

Table 11. *Continued.*

Alcohol denat.	Alcohol with a small amount of a denaturant is added to it (Cosmetics Info 2016a).
Acrylates/octylacrylamide copolymer	Film-forming, water-resistant polymers commonly used in sunscreens (Rigano 2014)
Bht	A phenolic antioxidant that reduces oxidative degradation (United States Department of Health, Education, and Welfare 1979)
Butyl methoxydibenzoylmethane	Most commonly used UVA absorber that protects over the whole UVA spectrum and then convert it to less damaging infrared radiation (Niculae et al. 2012; Cosmetics Info 2016g).
Ethylhexyl methoxycinnamate (EMC)	One of the most frequently used UVB filters in sunscreen products due to its high absorption capacity in the shorter wavelength region (Durand et al. 2010; Fevola 2012)
Propylene glycol	A synthetic organic alcohol that absorbs or attracts water (Cosmetics Info 2016m)
d&c orange no. 4 (cl 15510)	A synthetic pigment used as a color additive (Cosmetics Info 2016l, Food and Drug Administration 2017)
ext. d&c violet no. 2 (cl60730)	A synthetic colorant used as a color additive (Cosmetics Info 2016i, Food and Drug Administration 2017)
fd&c red no. 4 (cl 14700)	A synthetic colorant used as a color additive (Cosmetics Info 2016n, Food and Drug Administration 2017)
fd&c yellow no. 5 (cl 19140)	A synthetic pigment also known as tartrazineto used to impart color (Cosmetics Info 2016p, Food and Drug Administration 2017)

Table 12. *Calvin Klein's Men Obsession chemical composition (listed by Calvin Klein®).*

Chemical Name	Chemical Composition
Natural Components	
Cinnamal (3-phenyl-2-propenal)	The main component of cinnamon oil (Ackermann et al. 2009)
Cinnamyl alcohol	A component found in cinnamon oil (Ackermann et al. 2009)
Citral	Naturally occurs in the essential oils of the May Chang (*Litsea cubeba*) also known as the mountain pepper (Yu Li et al. 2014)
Citronellol	Industrially it is an important essential oil found in catnip and lemon grass (*Cymbopogan citratus*) (Resende et al. 2011; Piaru et al. 2012; Kakaraparthi et al. 2014)
Eugenol	Found in cinnamon oil one of its many components (Ackermann et al. 2009)
Farnesol	An acyclic sesquiterpene frequently used to emphasize the odors of scents and is widely distributed in many essential oils such as citronella, neroli, cyclamen, lemon grass, tuberose, rose, musk, and balsam (Ham 2013)
Geraniol	Found naturally in lemongrass (Fisher and Phillips 2008)
Hexyl cinnamal	A naturally occurring and synthetic scent component (EWG Skin Deep Cosmetics Database 2016)
Limonene	Found in citrus oils and considered is volatile in nature (Fisher and Phillips 2008)
Linalool	Found in many citrus oils and is the main part extracted from the orange blossom (Fisher and Phillips 2008)
Aqua/water/eau	Nontoxic water
Synthetic Components	
Benzyl cinnamate	A fragrance element used in numerous fragrance compounds (Bhatia et al. 2007)
Hydrolyzed jojoba esters	Derived from a reaction of oil from the jojoba plant (*Simmondsia chinensis*) and potassium hydroxide (Meyer et al. 2008)
Alcohol denat.	Alcohol with a small amount of a denaturant is added to it (Cosmetics Info 2016a).
Alpha-isomethyl ionone	A synthetic pale straw-colored or colorless liquid (Cosmetics Info 2016b)
Acrylates/octylacrylamide copolymer	Film-forming, water-resistant polymers commonly used in sunscreens (Rigano 2014)

Table 13. *Continued.*

Benzophenone-3	A sunscreen agent powder commonly used in various cosmetic products to protect cosmetics from deterioration by absorbing, or reflecting UV rays (Cosmetics Info 2016d; Yang and Ying 2013).
Benzyl benzoate	An ester of benzyl alcohol and benzoic acid (Cosmetics Info 2016e).
Bht	A phenolic antioxidant that reduces oxidative degradation (United States Department of Health, Education, and Welfare 1979)
Butyl methoxydibenzoylmethane	Most commonly used UVA absorber that protects over the whole UVA spectrum and then convert it to less damaging infrared radiation (Niculae et al. 2012; Cosmetics Info 2016g).
Ethylhexyl methoxycinnamate (EMC)	One of the most frequently used UVB filters in sunscreen products due to its high absorption capacity in the shorter wavelength region (Durand et al. 2010; Fevola 2012)
Ethylhexyl salicylate	An ester of salicylic acid and UV absorber (Mortz et al. 2010; Cosmetics Info 2016o).
Propylene glycol	A synthetic organic alcohol that absorbs or attracts water (Cosmetics Info 2016m)
d&c orange no. 4 (cl 15510)	A synthetic pigment used as a color additive (Cosmetics Info 2016l, Food and Drug Administration 2017)
ext. d&c violet no. 2 (cl60730)	A synthetic colorant used as a color additive (Cosmetics Info 2016i, Food and Drug Administration 2017)
fd&c red no. 4 (cl 14700)	A synthetic colorant used as a color additive (Cosmetics Info 2016n, Food and Drug Administration 2017)
fd&c yellow no. 5 (cl 19140)	A synthetic pigment also known as tartrazineto used to impart color (Cosmetics Info 2016p, Food and Drug Administration 2017)

Table 14. *Chemical composition of Yves Saint Laurent Opium (listed by Yves Saint Laurent®).*

Chemical Name	Chemical Composition
Natural Components	
Cinnamal (3-phenyl-2-propenal)	The main component of cinnamon oil (Ackermann et al. 2009)
Cinnamyl alcohol	A component found in cinnamon oil (Ackermann et al. 2009)
Citral	Naturally occurs in the essential oils of the May Chang (*Litsea cubeba*) also known as the mountain pepper (Yu Li et al. 2014)
Coumarin	Found in the tonka bean, it is a fermented substance that has a vanilla identical aroma and is used as a vanilla substitute (Columbia Encyclopedia 2013).
Geraniol	Found naturally in lemongrass (Fisher and Phillips 2008)
Hexyl cinnamal	A naturally occurring and synthetic scent component (EWG Skin Deep Cosmetics Database 2016)
Hydroxycitronellal	A pale yellow liquid that naturally occurs in some plants such as lavender with a sweet floral aroma (Cosmetics Info 2016j).
Isoeugenol	A natural chemical in ylang-ylang plant oil, it is pale yellow liquid with a zesty, carnation-like odor (Srivastava and Bajaj, 2014; Cosmetics Info 2016k)
Limonene	Found in citrus oils and considered is volatile in nature (Fisher and Phillips 2008)
Linalool	Found in many citrus oils and is the main part extracted from the orange blossom (Fisher and Phillips 2008)
Alcohol denat.	Alcohol with a small amount of a denaturant is added to it (Cosmetics Info 2016a).
Amyl cinnamal	A mostly synthetic a pale yellow liquid with a floral smell reminiscent of jasmine (Cosmetics Info 2016c)
Aqua/water/eau	Nontoxic water
Synthetic Components	
Benzyl alcohol	A primary alcohol and is a member of the fragrance structural group Aryl Alkyl Alcohols (Scognamiglio et al. 2012)
Benzyl salicylate	A clear colorless liquid that is an ultraviolet light absorber with a sweet floral aroma (Cosmetics Info 2016f)
Benzyl benzoate	An ester of benzyl alcohol and benzoic acid (Cosmetics Info 2016e).
Benzyl cinnamate	A fragrance element used in numerous fragrance compounds (Bhatia et al. 2007)
3yco4-3	Unknown

Table 15. *Chemical composition of Ralph Lauren Double Black (listed by Ralph Lauren®).*

Chemical Name	Chemical Composition
Natural Components	
Citral	Naturally occurs in the essential oils of the May Chang (*Litsea cubeba*) also known as the mountain pepper (Yu Li et al. 2014)
Citronellol	Industrially it is an important essential oil found in catnip and lemon grass (*Cymbopogan citratus*) (Resende et al. 2011; Piaru et al. 2012; Kakaraparthi et al. 2014)
Coumarin	Found in the tonka bean, it is a fermented substance that has a vanilla identical aroma and is used as a vanilla substitute (Columbia Encyclopedia 2013).
Geraniol	Found naturally in lemongrass (Fisher and Phillips 2008)
Hexyl cinnamal	A naturally occurring and synthetic scent component (EWG Skin Deep Cosmetics Database 2016)
Isoeugenol	A natural chemical in ylang-ylang plant oil, it is pale yellow liquid with a zesty, carnation-like odor (Srivastava and Bajaj 2014; Cosmetics Info 2016k)
Limonene	Found in citrus oils and considered is volatile in nature (Fisher and Phillips 2008)
Linalool	Found in many citrus oils and is the main part extracted from the orange blossom (Fisher and Phillips 2008)
Amyl cinnamal	A mostly synthetic a pale yellow liquid with a floral smell reminiscent of jasmine (Cosmetics Info 2016c)
Aqua/water	Nontoxic water
Synthetic Components	
Alcohol denat.	Alcohol with a small amount of a denaturant is added to it (Cosmetics Info 2016a).
Benzyl alcohol	A primary alcohol and is a member of the fragrance structural group Aryl Alkyl Alcohols (Scognamiglio et al. 2012)
Alpha-isomethyl ionone	A synthetic pale straw-colored or colorless liquid (Cosmetics Info 2016b)
D29099/1	Unknown
645335	Unknown

Table 16. *Chemical composition of Paco Rabanne's 1 Million (listed by Paco Rabanne®).*

Chemical Name	Chemical Composition
Natural components	
Citral	Naturally occurs in the essential oils of the May Chang (*Litsea cubeba*) also known as the mountain pepper (Yu Li et al. 2014)
Citronellol	Industrially it is an important essential oil found in catnip and lemon grass (*Cymbopogan citratus*) (Resende et al. 2011; Piaru et al. 2012; Kakaraparthi et al. 2014)
Coumarin	Found in the tonka bean, it is a fermented substance that has a vanilla identical aroma and is used as a vanilla substitute (Columbia Encyclopedia 2013).
Eugenol	Found in cinnamon oil one of its many components (Ackermann et al. 2009)
Hydroxycitronellal	A pale yellow liquid that naturally in some plants such as lavender with a sweet floral aroma (Cosmetics Info 2016j).
Isoeugenol	A natural chemical in ylang-ylang plant oil, it is pale yellow liquid with a zesty, carnation-like odor (Srivastava and Bajaj 2014; Cosmetics Info 2016k)
Limonene	Found in citrus oils and considered is volatile in nature (Fisher and Phillips 2008)
Linalool	Found in many citrus oils and is the main part extracted from the orange blossom (Fisher and Phillips 2008)
Aqua/water/eau	Nontoxic water
Synthetic Components	
Butylphenyl methylpropional	A synthetic colorless to pale yellow liquid with a powerful, floral-fresh odor (Cosmetics Info 2016h).
Alcohol denat.	Alcohol with a small amount of a denaturant is added to it (Cosmetics Info 2016a).
Alpha-isomethyl ionone	A synthetic pale straw-colored or colorless liquid (Cosmetics Info 2016b)
Benzyl alcohol	A primary alcohol and is a member of the fragrance structural group Aryl Alkyl Alcohols (Scognamiglio et al. 2012)

Table 17. *Chemical composition of Marc Jacob's Daisy (listed by Marc Jacobs®).*

Chemical Name	Chemical Composition
Natural Components	
Cinnamal (3-phenyl-2-propenal)	The main component of cinnamon oil (Ackermann et al. 2009)
Citronellol	Industrially it is an important essential oil found in catnip and lemon grass (*Cymbopogan citratus*) (Resende et al. 2011; Piaru et al. 2012; Kakaraparthi et al. 2014)
Geraniol	Found naturally in lemongrass (Fisher and Phillips 2008)
Hexyl cinnamal	A naturally occurring and synthetic scent component (EWG Skin Deep Cosmetics Database 2016)
Hydroxycitronellal	A pale yellow liquid that naturally in some plants such as lavender with a sweet floral aroma (Cosmetics Info 2016j).
Limonene	Found in citrus oils and considered is volatile in nature (Fisher and Phillips 2008)
Linalool	Found in many citrus oils and is the main part extracted from the orange blossom (Fisher and Phillips 2008)
Aqua/water/eau	Nontoxic water
Synthetic Components	
Hydrolyzed jojoba esters	Derived from a reaction of oil from the jojoba plant (*Simmondsia chinensis*) and potassium hydroxide (Meyer et al. 2008)
Methyl 2-0ctynoate	A synthetic fragrance (Heisterberg et al. 2010).
Acrylates/octylacrylamide copolymer	Film-forming, water-resistant polymers commonly used in sunscreens (Rigano 2014)
Alcohol denat.	Alcohol with a small amount of a denaturant is added to it (Cosmetics Info 2016a).
Alpha-isomethyl ionone	A synthetic pale straw-colored or colorless liquid (Cosmetics Info 2016b)
Benzophenone-3	A sunscreen agent powder commonly used in various cosmetic products to protect cosmetics from deterioration by absorbing, or reflecting UV rays (Yang and Ying 2013; Cosmetics Info 2016d).
Benzyl benzoate	An ester of benzyl alcohol and benzoic acid (Cosmetics Info 2016e).
Bht	A phenolic antioxidant that reduces oxidative degradation (United States Department of Health, Education, and Welfare 1979)

Table 18. *Continued.*

Butyl methoxydibenzoylmethane	Most commonly used UVA absorber that protects over the whole UVA spectrum and converts it to less harmful radiation (Niculae et al. 2012; Cosmetics Info 2016g).
Ethylhexyl methoxycinnamate	One of the most frequently used UVB filters in sunscreen products due to its high absorption capacity in the shorter wavelength region (Durand et al. 2010; Fevola 2012)
Ethylhexyl salicylate	An ester of salicylic acid and UV absorber (Mortz et al. 2010; Cosmetics Info 2016o).
Propylene glycol	A synthetic organic alcohol that absorbs or attracts water (Cosmetics Info 2016m)
fd&c yellow no. 5 (cl 19140)	A synthetic pigment also known as tartrazineto used to impart color (Cosmetics Info 2016p, Food and Drug Administration 2017)
fd&c yellow no. 6 (cl 15985)	is a synthetic pigment sometimes referred to as a monoazo color (Cosmetics Info 2016q, Food and Drug Administration 2017)

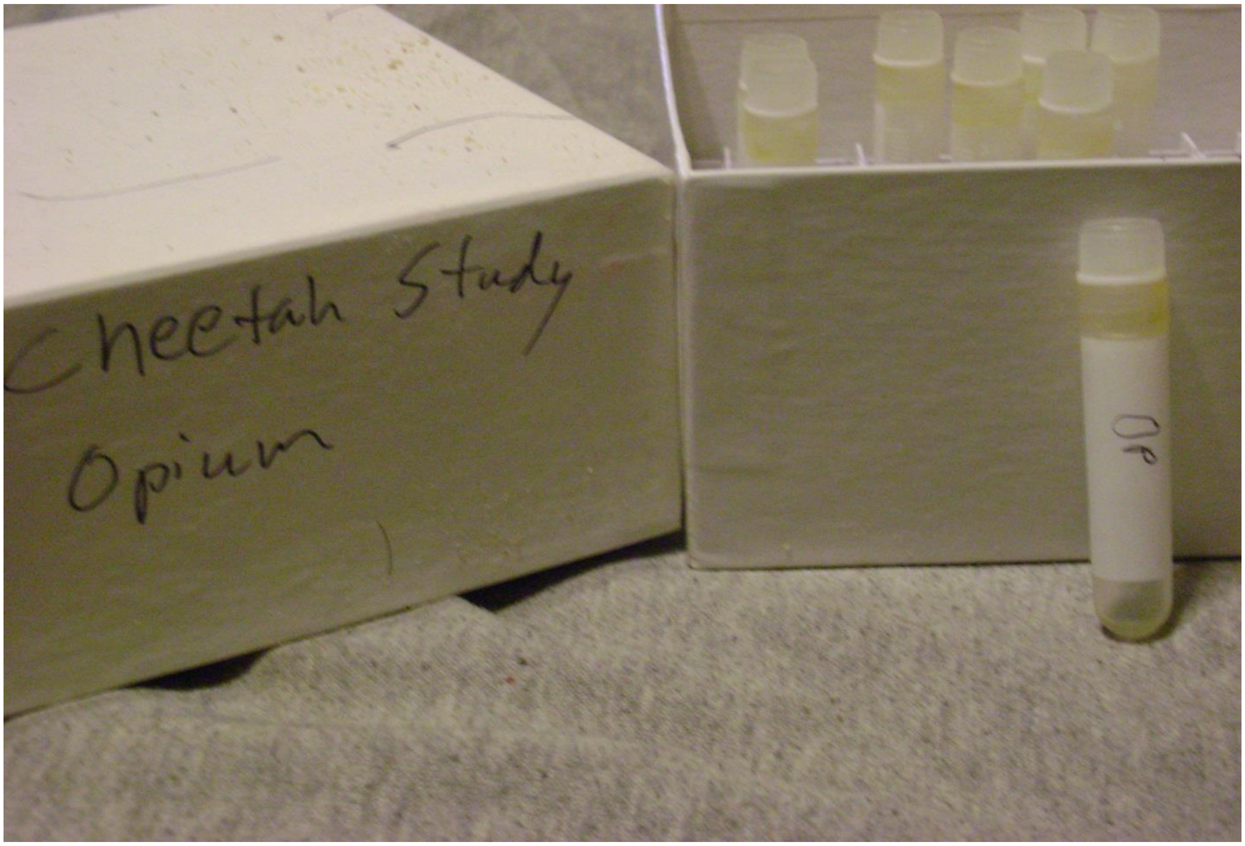


Figure 1. Labeled, aliquoted fragrance and containment box

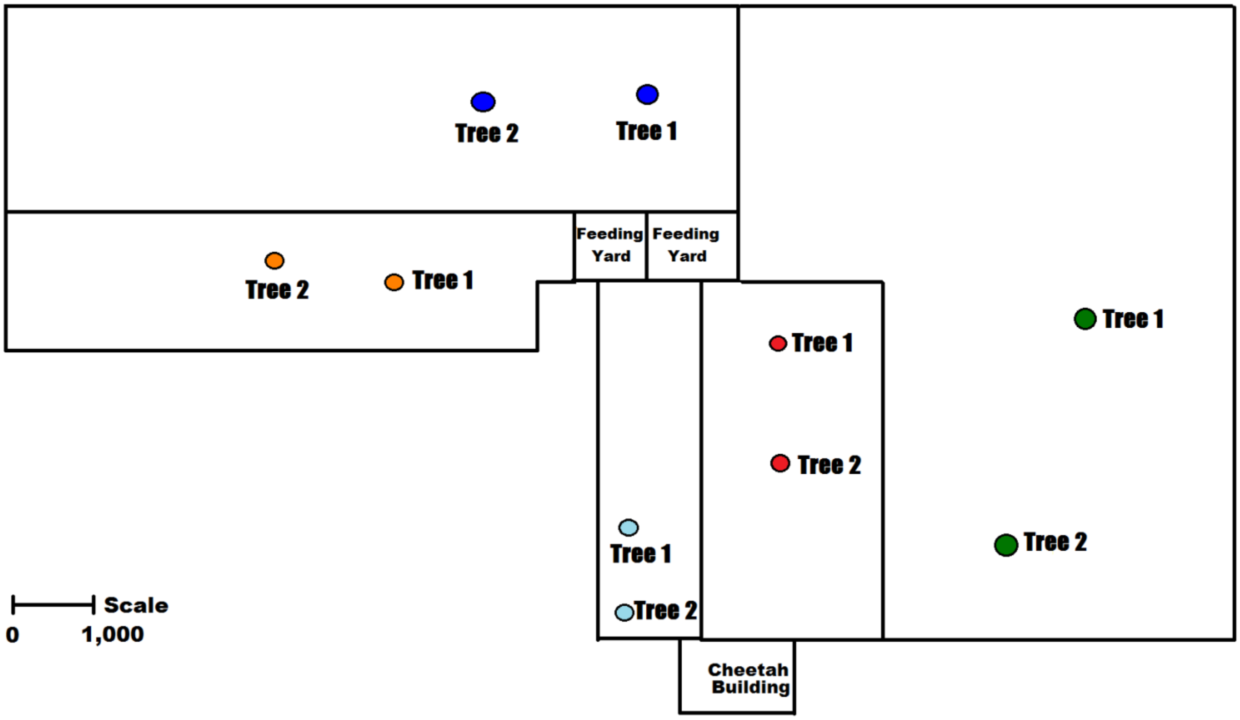
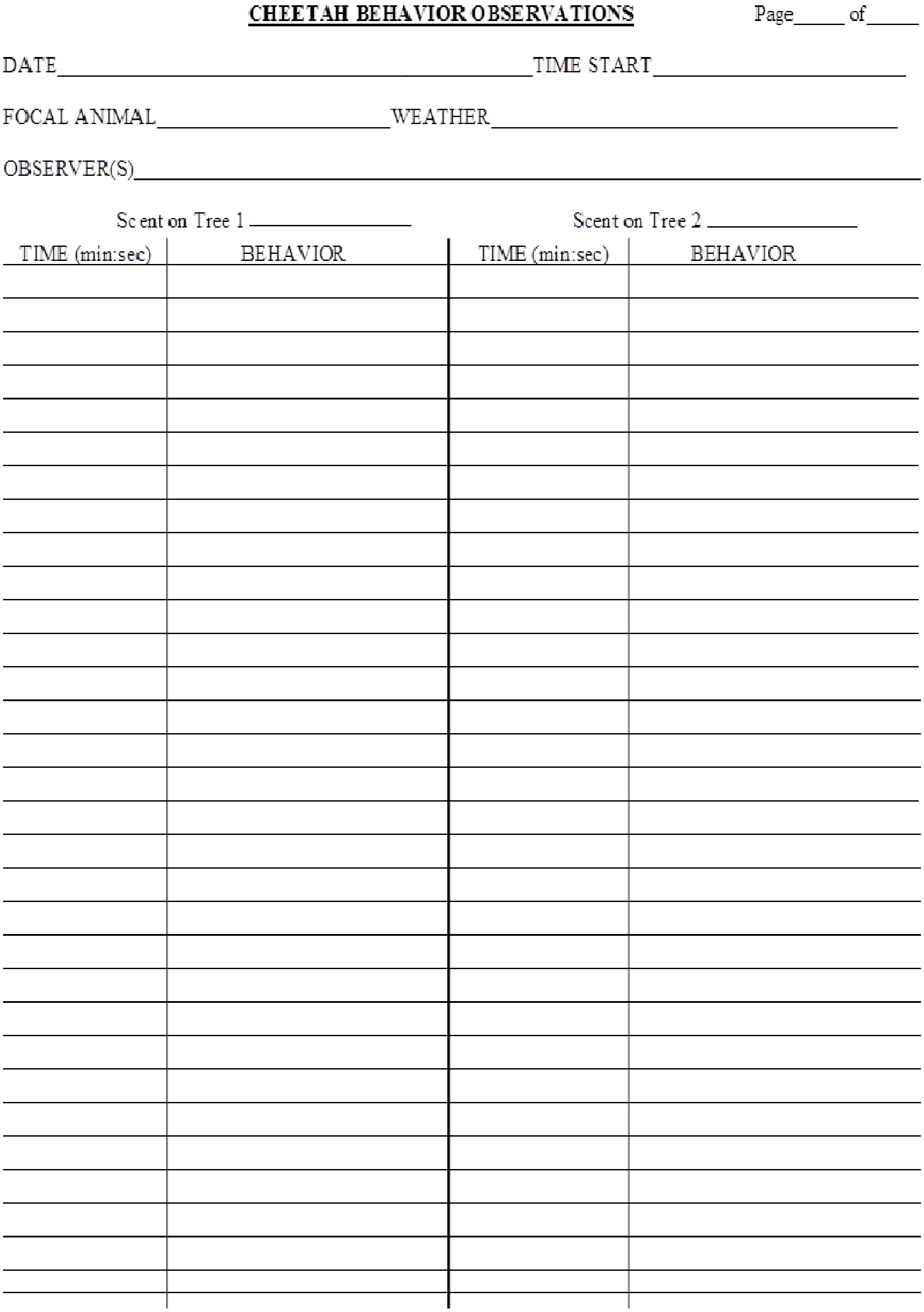


Figure 2. African cheetah exhibit design in the St. Louis Zoo.

Figure 3. Cheetah scent dispenser.

Figure 4. Cheetah scent dispenser in place for testing.



CHEETAH BEHAVIOR OBSERVATIONS Page_____ of_____

DATE____TIME START_______________________

FOCAL ANIMAL_____________________WEATHER_____________________________________

OBSERVER(S)____

Scent on Tree 1 _______________ Scent on Tree 2 ______________

TIME (min:sec)	BEHAVIOR	TIME (min:sec)	BEHAVIOR

Figure 5. Cheetah behavior sheet used to record data.

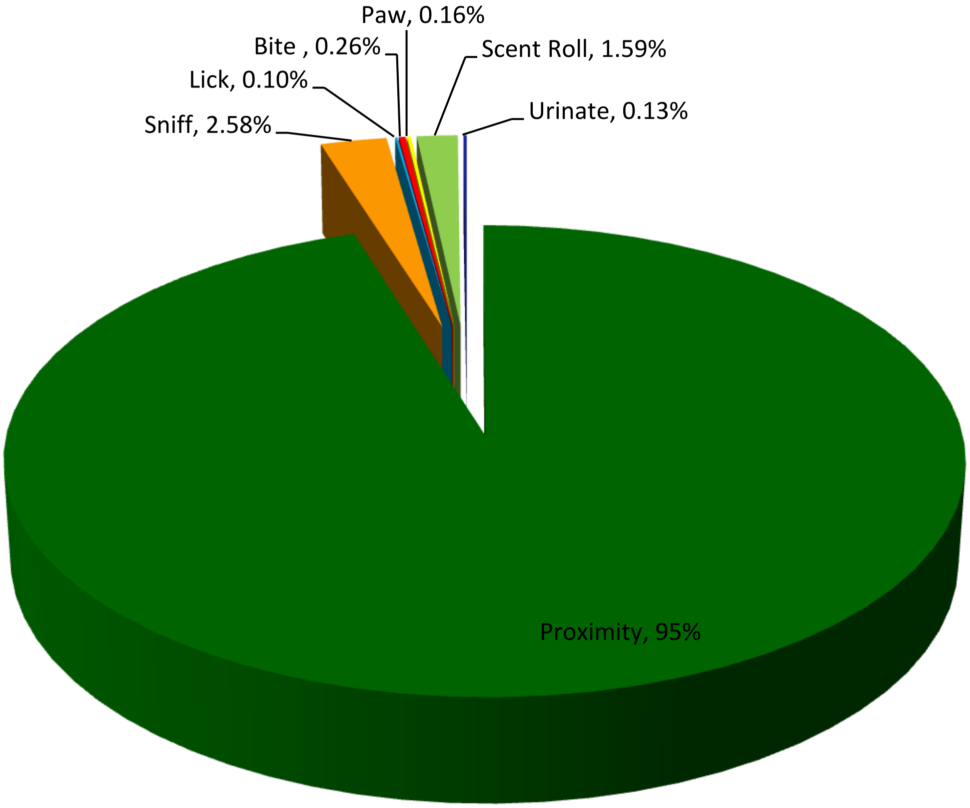


Figure 6. Detailed description of Jason's behavioral responses to all scents; percentages are based on 51 total minutes and 14 seconds (4.84%) of interaction during 1080 minutes of observation.

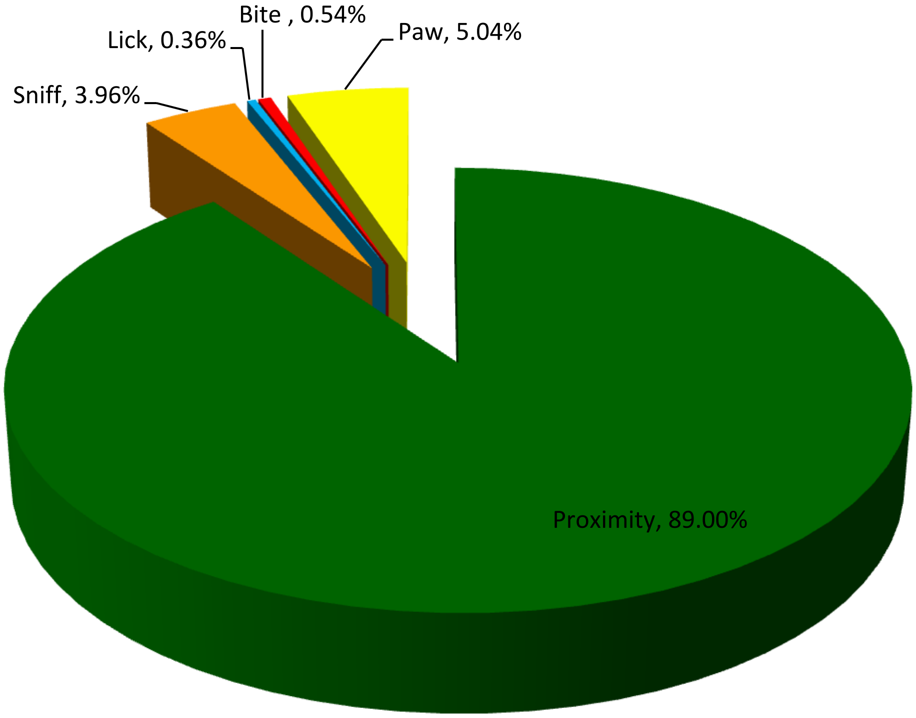


Figure 7. Detailed description of Zuri’s behavioral responses to all scents; percentages are based on 9 total minutes and 16 seconds (0.85%) of interaction during 1080 minutes of observation.

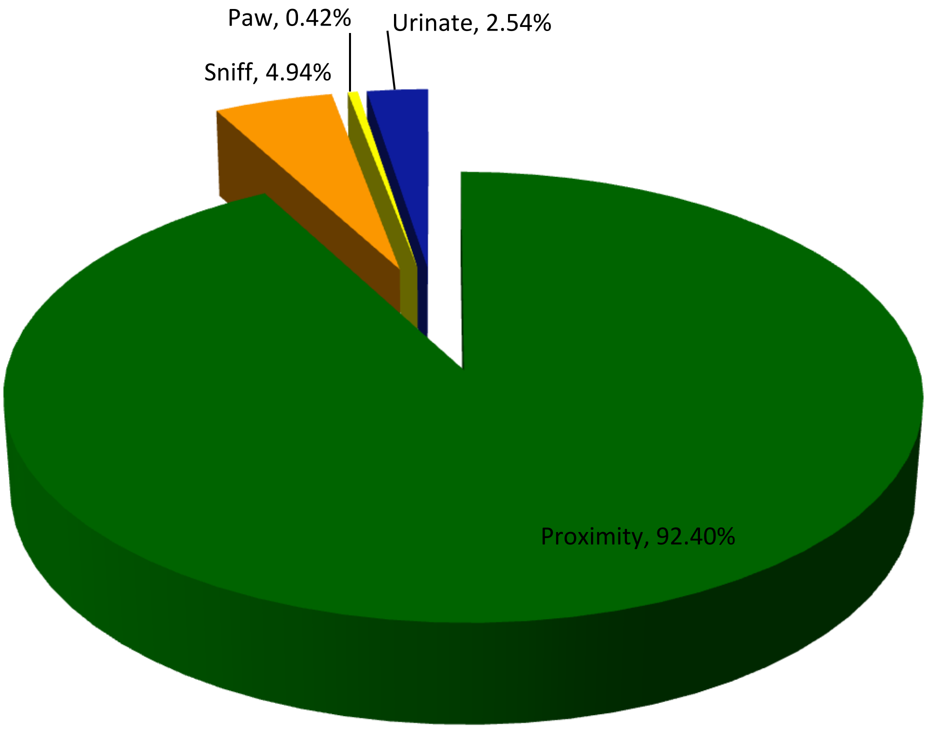


Figure 8. Detailed description of Shanto's behavioral responses to all scents; percentages are based on 4 total minutes and 23 seconds (0.41%) of interaction during 1080 minutes of observation.

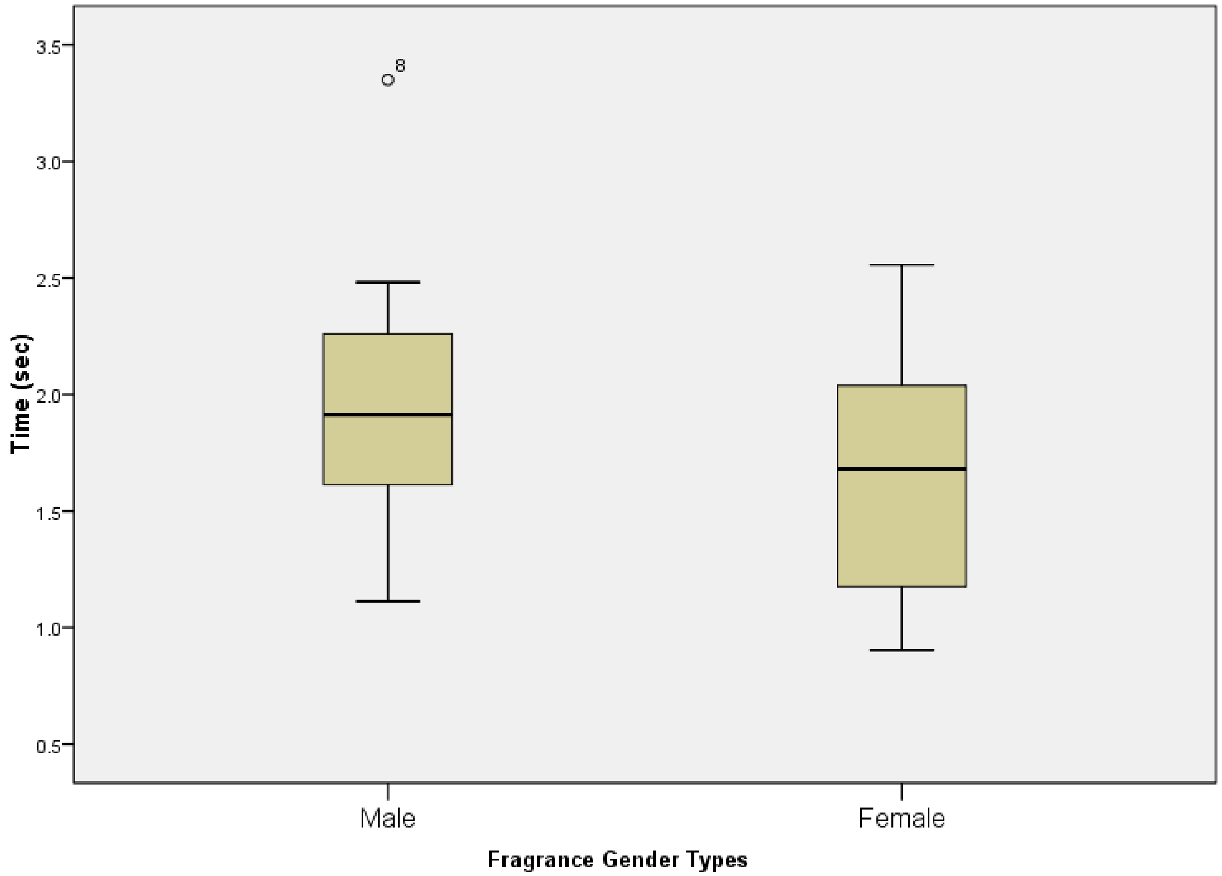


Figure 9. Box plots of $\log_{10}$-transformed interaction times of cheetahs with male and female fragrances. The circle represents the outlier. Whiskers (black bars) indicate ranges excluding the outlier.

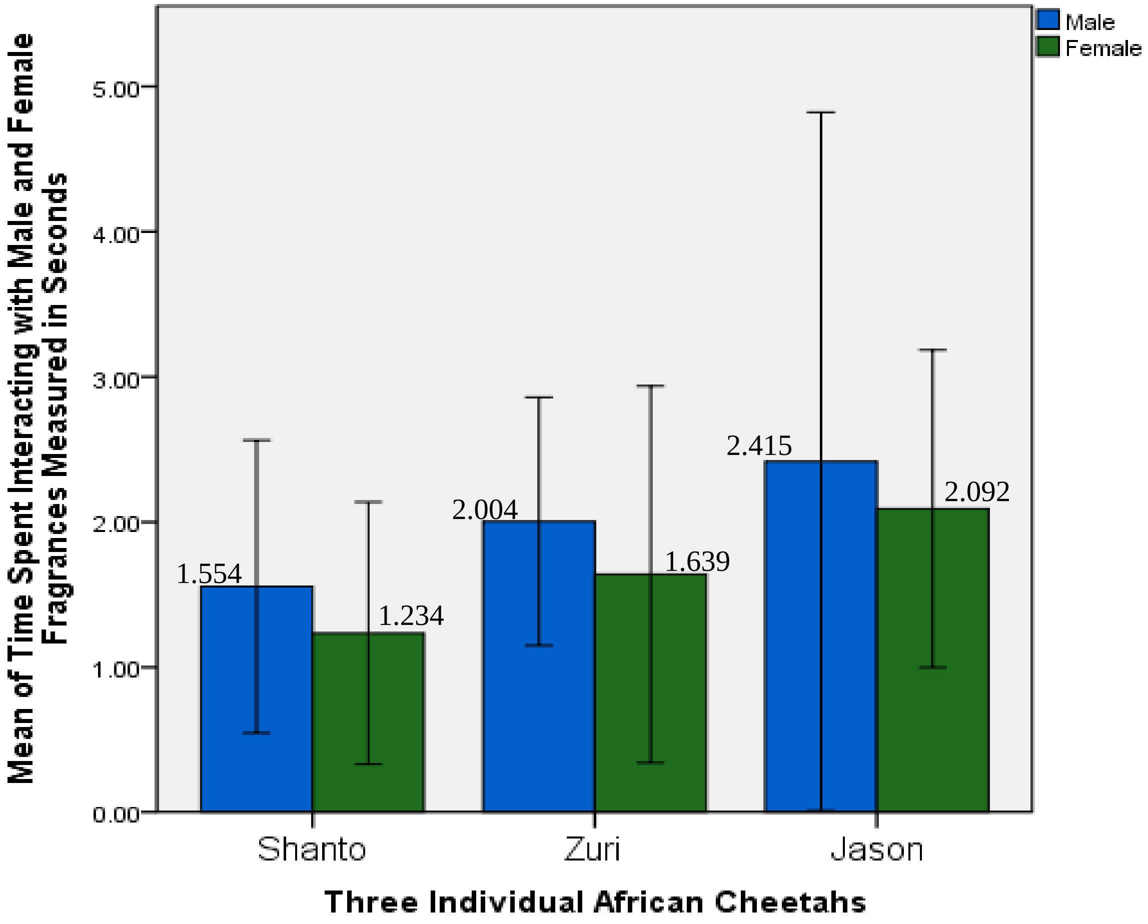


Figure 10. Means detailing African cheetah interaction times ($\log_{10}$-transformed seconds) with male and female fragrances. Error bars: 95% CI. The fourth cheetah was excluded because he provided no data.

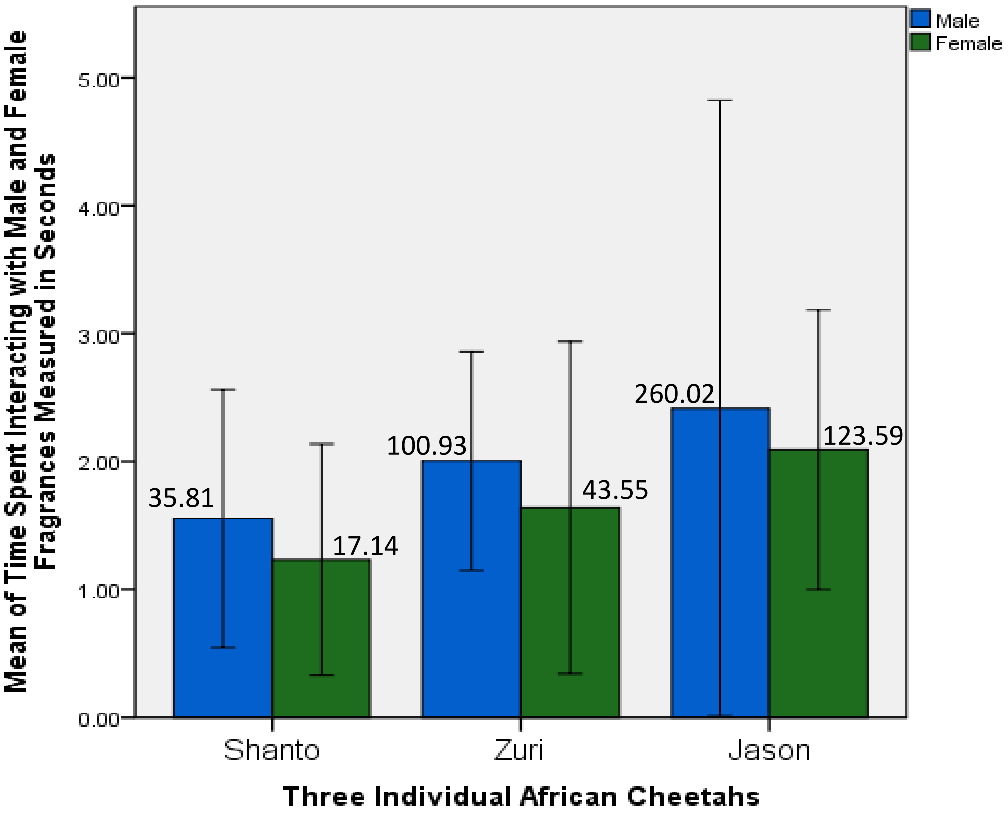


Figure 11. Means detailing African cheetah interaction times (untransformed seconds) with male and female fragrances. Error bars: 95% CI. The fourth cheetah was excluded because he provided no data.

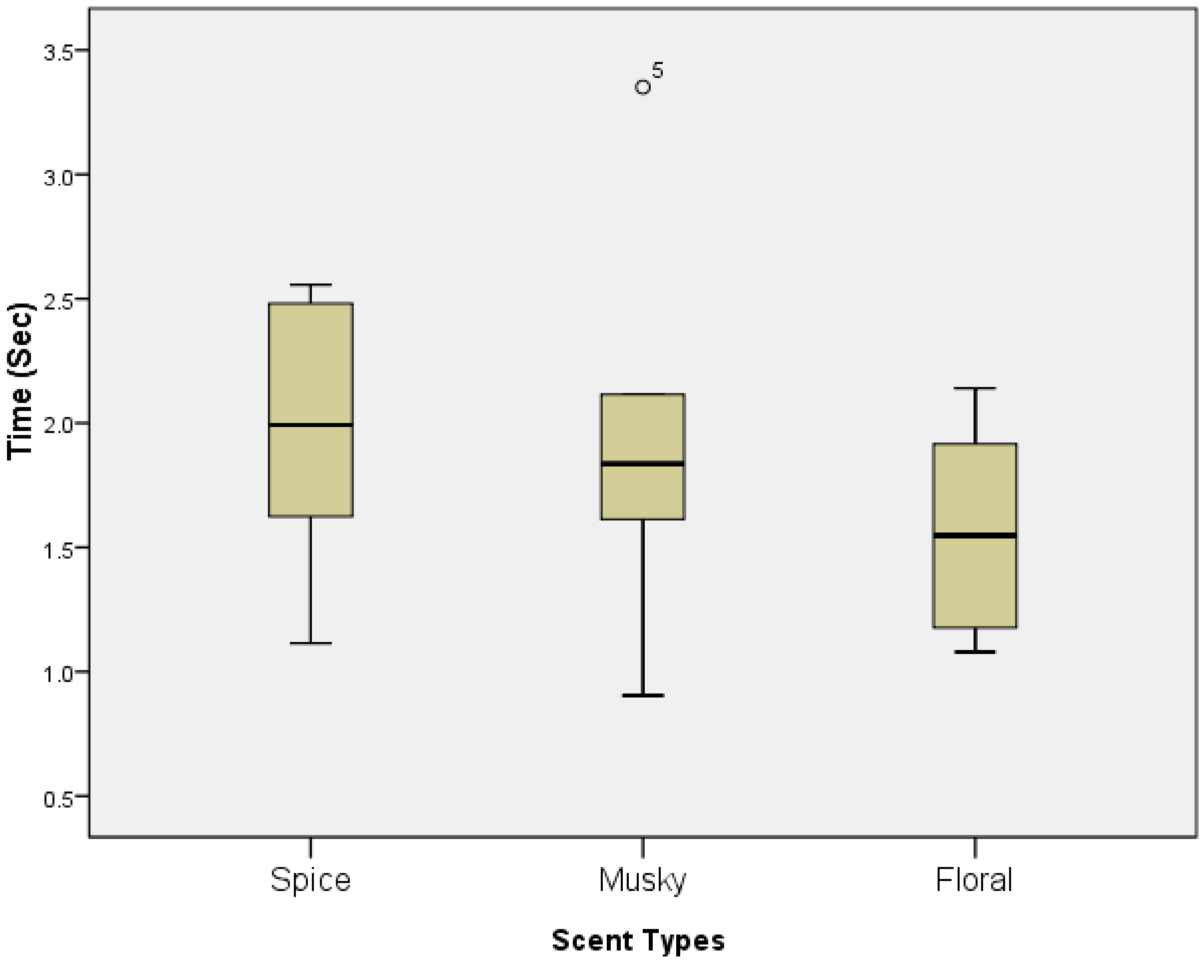


Figure 12. Box plots of ($\log_{10}$-transformed seconds) interaction times with scent types. The circle represents an outlier. Whiskers indicate ranges excluding the outlier.

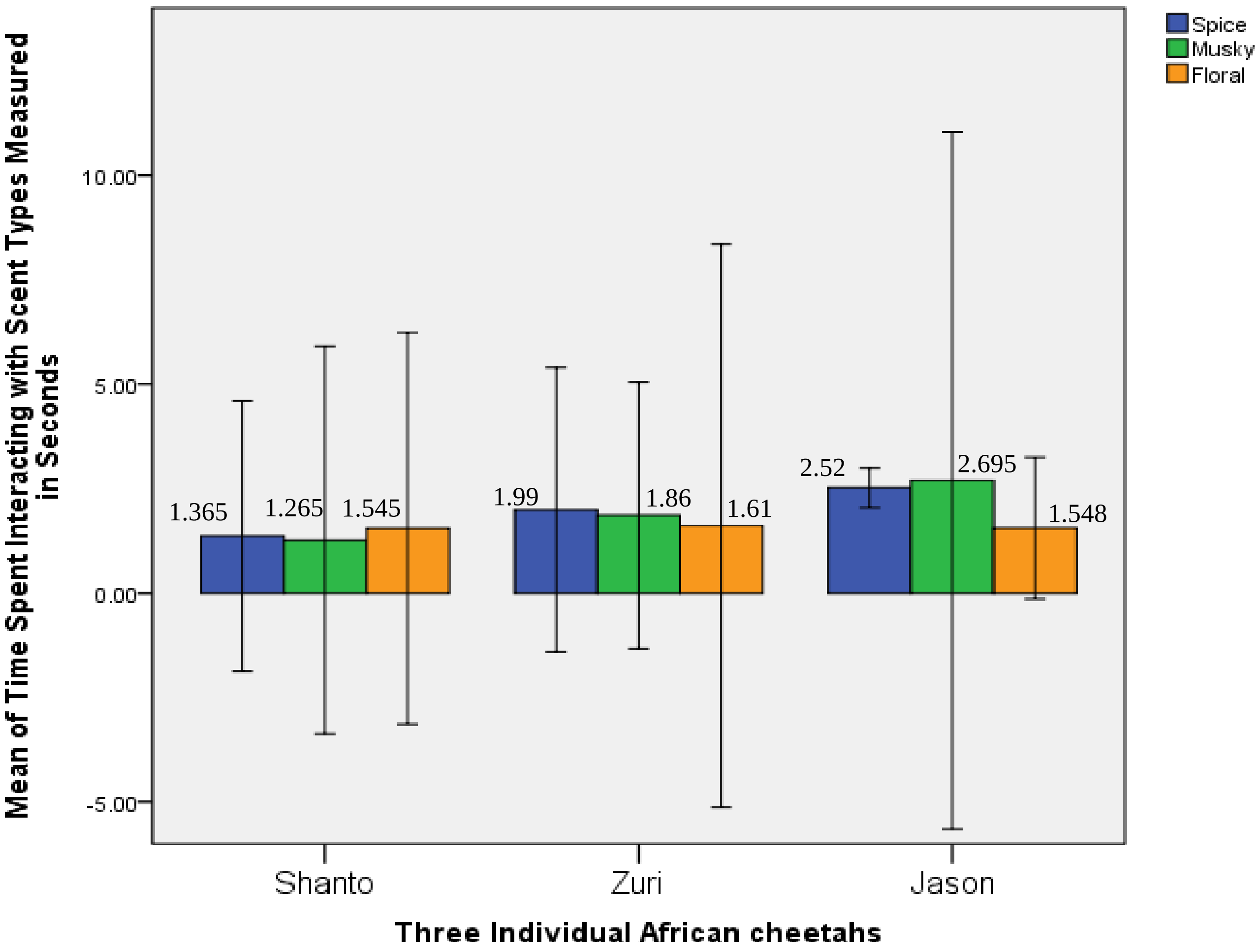


Figure 13. Comparisons for the amount of time ($\log_{10}$-transformed seconds) African cheetahs spent with scent types. The means and 95% CI's of each individual response are displayed. The fourth cheetah was excluded because he provided no data.

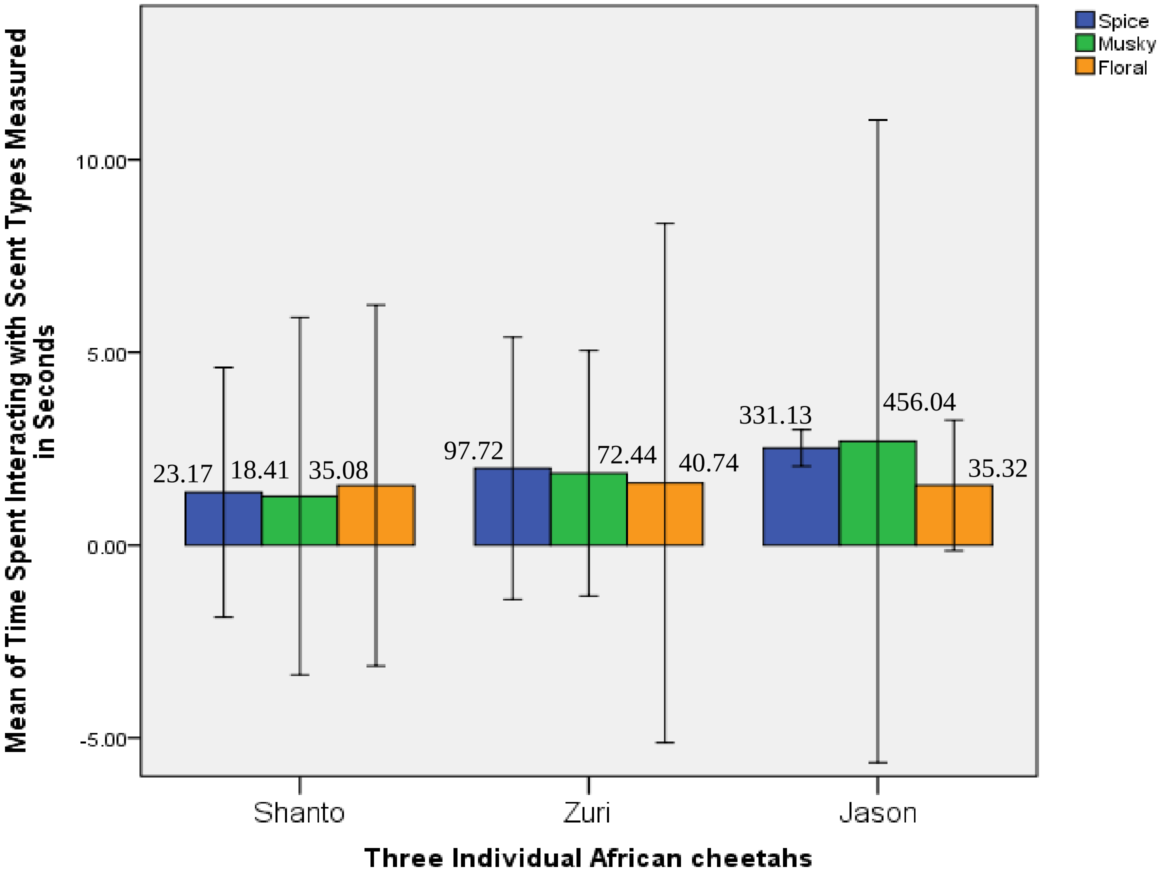


Figure 14. Comparisons for the amount of time (untransformed seconds) African cheetahs spent with scent types. The means and 95% CI's of each individual response are displayed. The fourth cheetah was excluded because he provided no data.

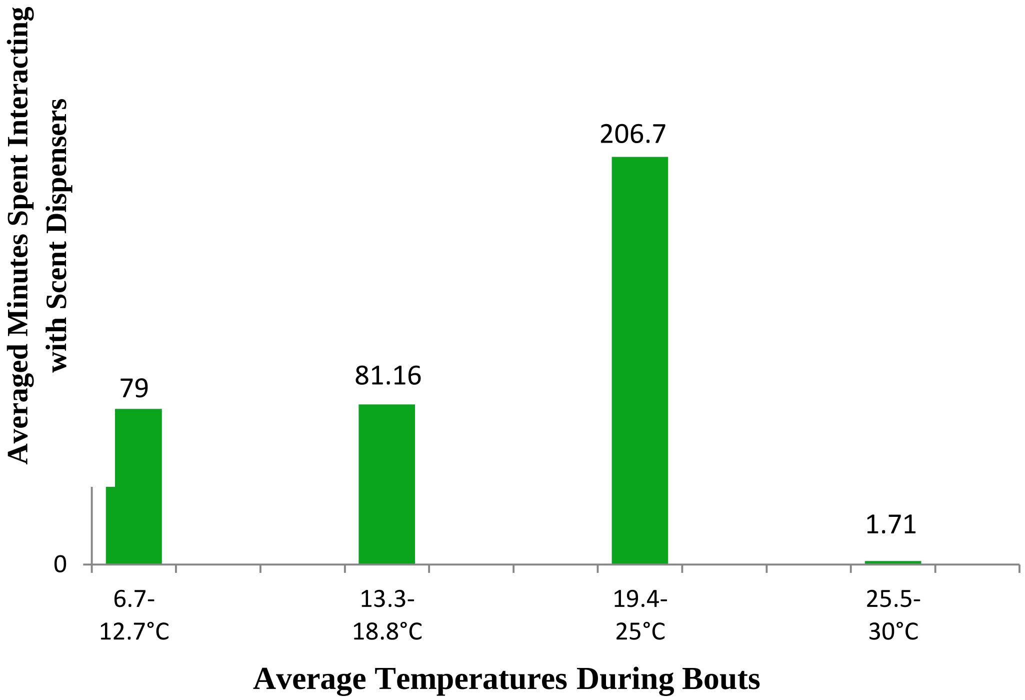


Figure 15. Average number of minutes cheetahs spent interacting with scent dispensers at four ambient temperature ranges.

Figure 16. Jason at the Saint Louis Zoo, photography is by Chris Carter**.**

Figure 17. Zuri with enrichment, photography credit belongs to the Saint Louis Zoo.

Figure 18. Shanto at the Saint Louis Zoo, photography is by Chris Carter.

Figure 19. Kgosi at the Saint Louis Zoo, photography credit goes to Chris Carter.

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APPENDICES

APPENDIX A

RAW CHEETAH DATA

The fragrance codes are PDB (Ralph Lauren Double Black®), Opium (Yves Saint Laurent Opium), CKOM (Men's Calvin Klein Obsession®), CKOB (Women's Calvin Klein Obsession®), PRM (Paco Rabanne's 1 Million®), and MJD (Marc Jacob's Daisy®)

Detailed data for every bout involving Zuri. Behavioral Codes are Proximity (PR), Flehmen (F), Sniff (S), Scent Roll (SR), Lick (L), Urinate (U), Bite (B), Paw (P), and Hiss (H).

DATA STARTS FOR 2012

Time (min:sec) Male Fragrance	Behavior	Tree 1	Time (min:sec) Female Fragrance	Behavior	Tree 2	Weather	Date	Male/Female
:00	PR						**Day 1 Oct. 12**	
:01	S	Left	:00	PR	Right	16.66°C, Sunny		PDB/MJD
:01	P		:12	PR (ends)				
:01	S							
:11	PR (ends)							
Total Time			**Total Time**					
11 sec			12 sec					
						Day 2 Oct. 26		
								PRM/Opium
:00	PR	Left	:00	PR	Right	6.66°C, Sunny		
:02	S		:01	S				
1:15	PR (ends)		:01	S				
			:27	PR (ends)				
			:00	PR				
			:01	P				
			:02	S				
			:01	B				
			:01	P				
			:01	P				
			:02	S				
			:01	P				
			:01	P				

			:01	P			
			:02	S			
			:01	P			
			:01	P			
			:01	B			
			1:06min	PR (ends)			
Total Time			**Total Time**				
1:15min			1:33min				
						Day 3 Oct. 27	
							PRM/CKOB
:00	PR	Right	0-30:00min	AI	Left	7.22°C, Sunny	
:02	PR (ends)						
Total Time			**Total Time**				
2 secs			0				
						Day 4 Oct. 28	
							PRM/MJD
:00	PR	Left	0-30:00min	AI	Right	6.66°C, Windy Sunny	
:01	P						
:27secs	PR (ends)						
Total Time			**Total Time**				
27secs			0				
							Day 5 Nov. 3
							CKOM/MJD
0-30:00min	AI	Left	0-30:00min	AI	Right	9.44°C, Cloudy	
Total Time			**Total Time**				
0			0				

							Day 6 Nov. 4
						CKOM/Opium	
0-30:00min	AI	Right	0-30:00min	AI	Left	6.66°C, Sunny	
Total Time			**Total Time**				
0			0				
							Day 7 Nov. 10
							PDB/Opium
:00	PR	Left	:00	PR	Right	9.44°C, Cloudy, Sprinkled	
:02	PR (ends)		:01	P			
:00	PR		:07	PR (ends)			
:01	S						
:01	P						
:01	P						
:02	P						
:10	PR (ends)						
Total Time			**Total Time**				
12 secs			7 secs				
							Day 8 Nov. 11
							PDB/CKOB
0-30:00min	AI	Right	0-30:00min	AI	Left	17.22°C, Cloudy	
Total Time			**Total Time**				
0			0				
							Day 9 Nov. 16
							PRM/Opium
0-30:00min	AI	Left	0-30:00min	AI	Right	11.11°C, Sunny	

Total Time			**Total Time**				
0			0				
						Day 10 Nov. 17	
							PRM/CKOB
0-30:00min	AI	Right	0-30:00min	AI	Left	11.11°C, Sunny	
Total Time			**Total Time**				
0			0				
						Day 11 Nov. 30	
							CKOM/CKOB
0-30:00min	AI	Left	0-30:00min	AI	Right	14.44°C, Sunny	
Total Time			**Total Time**				
0			0				
						Day 12 Dec. 1	
0-30:00 min	AI	Right	0-30:00 min	AI	Left	14.44°C, Partly Sunny	CKOM/MJD
Total Time			**Total Time**				
0			0				
						Day 13 Dec. 2	
0-30:00 min	AI	Left	0-30:00 min	AI	Right	20°C, Cloudy	CKOM/Opium
Total Time			**Total Time**				
0			0				
						Day 14 Dec. 9	
:00	PR	Left	0-5 secs	PR	Right	9.44°C, Partly Sunny/Sprinkles	PDB/Opium
:02	PR (ends)		0:01	P		Special note: Zuri ran over to dispenser twice (play charge)	
:00	PR		7 secs	PR (ends)			
:01	S						
:01	P						

:01	P						
:01	P						
:10	PR (ends)						
Total Time			**Total Time**				
18 secs			7 secs				
			2013-2014 DATA STARTS				
							Day 15 May 11
0-30:00 min	AI	Left	0-30:00 min	AI	Right	21.11°C, Sunny	PRM/OPIUM
Total Time			**Total Time**				
0			0				
							Day 16 June 1
0-30:00 min	AI	Right	0-30:00 min	AI	Left	20°C, Sunny	PDB/MJD
Total Time			**Total Time**				
0			0				
							Day 17 June 2
0-30:00 min	AI	Left	0-30:00 min	AI	Right	20°C, Partly Sunny	PDB/CKOB
Total Time			**Total Time**				
0			0				
							Day 18 June 23
0-30:00 min	AI	Left	0-30:00 min	AI	Right	23.88°C, Partly Sunny	PRM/MJD
Total Time			**Total Time**				
0			0				
							Day 19 June 30
0-30:00 min	AI	Right	0-30:00 min	AI	Left	22.22°C, Cloudy, Drizzle	CKOM/CKOB

Total time			**Total Time**				
0			0				
							Day 20 July 6
							PRM/CKOB
0-30:00 min	AI	Left	0-30:00 min	AI	Right	28.88°C, Partly Cloudy	
Total time			**Total Time**				
0			0				
							Day 21 July 7
0-30:00 min	AI	Left	0-30:00 min	AI	Right	30°C, Sunny	CKOM/MJD
Total Time			**Total Time**				
0			0				
							Day 22 July 13
0-30:00 min	AI	Right	0-30:00 min	AI	Left	30°C, Sunny	PRM/MJD
Total Time			**Total Time**				
0			0				
							Day 23 July 14
0-30:00 min	AI	Right	0-30:00 min	AI	Left	30°C, Sunny	CKOM/CKOB
Total Time			**Total Time**				
0			0				
							Day 24 July 28
0-30:00 min	AI	Left	0-30:00 min	AI	Right	23.88°C, Sunny	CKOM/Opium
Total Time			**Total Time**				
0			0				
							Day 25 Aug. 4
0-30:00 min	AI	Right	0-30:00 min	AI	Left	25.55°C, Partly Sunny	PDB/Opium
Total			**Total Time**				

Time							
0			0				
							Day 26 Aug. 11
0-30:00 min	AI	Right	0-30:00 min	AI	Left	25.55°C, Partly Sunny	PDB/MJD
Total Time			**Total Time**				
0			0				
							Day 27 Aug. 18
0-30:00 min	AI	Left	0-30:00 min	AI	Right	25.55°F, Partly Sunny	PDB/CKOB
Total Time			**Total Time**				
0			0				
							Day 28 Sept. 22
:00	PR	Right	:00	PR	Left	22.22°C, Sunny	PRM/Opium
:01	S		:01	S			
:01	S		:01	P			
:34 secs	PR (ends)		:23 secs	PR (ends)			
Total Time			**Total Time**				
34 secs			23 secs				
							Day 29 Sept 29
0-30:00 min	AI	Left	:00	PR	Right	20°C, Sunny	PRM/CKOB
			:01	S			Special note: Zuri picked up dispenser with her paw.
			:01	P			
			:01	P			
			:02	S			
			:03	P			
			:01	P			
			:01	S			
			:01	S			
			:53	PR (ends)			

Total Time			**Total Time**				
0 secs			53 secs				
							Day 30 Oct. 20
0-30:00 min	AI	Right	0-30:00 min	AI	Left	14.44°C, Sunny	PRM/MJD
Total Time			**Total Time**				
0 secs			0 secs				
							Day 31 Oct. 27
:00	PR	Right	0-30:00 min	AI	Left	10.55°C, Sunny	CKOM/CKOB
:01	P						
:01	P						
:01	P						
:02	L						
:01	B						
:01	P						
3:02	PR (ends)						
Total Time			**Total Time**				
3:02 mins			0 secs				
							Day 32 Nov. 3
:00-30:00min	AI	Left	0-30:00 min	AI	Right	10°C, Partly Sunny	CKOM/Opium
Total Time			**Total Time**				
0			0 secs				
							Day 33 Nov. 10
:00-30:00min	AI	Right	0-30:00 min	AI	Left	10.55°C, Partly Sunny	CKOM/MJD
Total Time			**Total Time**				
0			0 secs				
							Day 34 Nov. 17
:00-30:00min	AI	Right	0-30:00 min	AI	Left	22.77°C, Sunny, Rained after	PDB/CKOB

						bout	
Total Time			**Total Time**				
0			0 secs				
							Day 35 Nov. 30
:00-30:00min	AI	Left	0-30:00 min	AI	Right	9.44°C Sunny	PDB/MJD
Total Time			**Total Time**				
0			0 secs				
							Day 36 Dec. 1
:00-30:00min	AI	Right	0-30:00 min	AI	Left	8.33°C Sunny	PDB/Opium
Total Time			**Total Time**				
0			0 secs				

Detailed data for every bout involving Jason. Behavioral Codes are Proximity (PR), Flehmen (F), Sniff (S), Scent Roll (SR), Lick (L), Urinate (U), Bite (B), Paw (P), and Hiss (H).

DATA STARTS FOR 2012

Time (min:sec) Male Fragrance	Behavior	Tree 1	Time (min:sec) Female Fragrance	Behavior	Tree 2	Weather	Date	Male/Female
							Day 1 Oct. 13	
0-30:00	AI	Right	0:00	PR	Left	18.88°C, Sunny	PRM/Opium	
			:02	S				
			:17	PR (ends)				
			0	PR				
			:02	S				
			:10	PR (ends)				
Total Time			**Total Time**					
0			31 sec					
							Day 2 Oct. 20	
0-30:00	AI	Left	:00	PR	Right	10°C, Cloudy, Sprinkles	PRM/CKOB	
			:01	S				
			:08	PR (ends)				
			:00	PR				
			:02	S				
			:32	PR (ends)				
			:00	PR				
			:01	S				
			:08	PR (ends)				
			:00	PR				
			:23	PR (ends)				
			:00	PR				
			:02	S				
			:08	PR (ends)				
Total Time			**Total Time**					
0			85 seconds					

							Day 3 Oct. 21	
0-30:00min	AI	Right	0-30:00min	AI	Left	11.11°C, Partly Cloudy		PRM/MJD
Total Time			**Total Time**					
0			0					
							Day 4 Oct. 26	
:00	PR	Left	:00	PR	Right	6.66°C, Sunny	CKOM/CKOB	
:16	SR		:04	PR (ends)				
:01	SR							
3:41	PR (ends)							
:00	PR							
:08	S							
:15	PR (ends)							
Total Time			**Total Time**					
3:56min			4 secs					
							Day 5 Oct. 27	
:00	PR	Right	0-30min	AI	Left	6.66°C, Sunny	CKOM/MJD	
:01	U							
:13	PR (ends)							
:00	PR							
:01	SR							
:21	PR (ends)							
	Total Time		**Total Time**					
	34 secs		0					
							Day 6 Nov. 2	
:00	PR	Left	:00	PR	Right	7.77°C, Partly Sunny		PDB/MJD
:01	S		:01	S				
:01	U		:01	S				
:01	U		:43	PR (ends)				
:31	PR (ends)							
Total Time			**Total Time**					
:31secs			43 secs					

							Day 7 Nov. 3	
0-30:00min	AI	Right	0-30:00min	AI	Left	9.44°C, Cloudy		PDB/Opium
Total Time			**Total Time**					
0			0					
							Day 8 Nov. 4	
0-30:00min	AI	Left	:00	PR	Right	6.66°C, Sunny		PDB/CKOB
			:02	S				
			:01	SR				
			4:00	PR (ends)				
Total Time			**Total Time**					
0			4:00 min					
							Day 9 Nov. 9	
0-30:00min	AI	Left	:00	PR	Right	18.88°C, Sunny	PRM/Opium	
			:03	PR (ends)				
Total Time			**Total Time**					
0			3 secs					
							Day 10 Nov. 10	
0-30:00min	AI	Right	0-30:00min	AI	Left	17.77°C, Sunny	PRM/CKOB	
Total Time			**Total Time**					
0			0					
							Day 11 Nov. 11	
0-30:00 min	AI	Left	:00	PR	Right	17.22°C, Cloudy		PRM/MJD
			:01	S				
			:05	PR (ends)				
Total Time			**Total Time**					
0			5 secs					

							Day 12 Nov. 16
:00	PR	Left	0-30:00 min	AI	Right	11.66°C, Sunny	CKOM/CKOB
:01	S						
:07	PR (ends)						

Total Time			**Total Time**				
7 secs			0				
							Day 13 Nov. 17
:00	PR	Right	0-30:00 min	AI	Left	11.11°C, Sunny	CKOM/MJD
:01	S						
:01	S						
:01	P						
:01	S						
:22	PR (ends)						

Total Time			**Total Time**				
22 secs			0				
							Day 14 Nov. 18
0-30:00 min	AI	Left	0-30:00 min	AI	Right	11.11°C, Sunny	CKOM/Opium

Total Time			**Total Time**				
0			0				
							Day 15 Nov. 30
:00	PR	Left	:00	PR	Right	14.44°C, Sunny	PDB/MJD
:02	S		:02	S			
:01	B		:01	U			
:01	L		:22	PR (ends)			
:01	B						
:01	P						
:01	P						
:01	B						
:01	B						
:01	B						
:01	P						
:37	S						
:01	SR						

:01	SR							
:01	SR							
:01	SR							
:01	SR							
6:31	PR (ends)							
Total Time			**Total Time**					
6:31 min			22 secs					
							Day 16 Dec. 1	
0-30:00 min	AI	Right	0-30:00 min	AI	Left	14.44°C, Partly Sunny		PDB/Opium
Total Time			**Total Time**					
0			0					
							Day 17 Dec. 2	
:00	PR	Left	:00	PR	Right	20°C, Cloudy	PDB/CKOB	
:02	S		:01	S				
:05	PR		:01	B				
			:01	B				
			:01	L				
			:01	B				
			:01	P				
			:15	PR (ends)				
			:00	PR				
			:01	S				
			:02	PR				
Total Time			**Total Time**					
:05 secs			17 secs					
			DATA SET STARTS FOR 2013-14					
							Day 18 May 19	
0	AI	Right	0	AI	Left	28.33°C, Partly Sunny		PDB/OPIUM
Total Time			**Total Time**					
0			0					
							Day 19 June 2	
:00	PR	Left	0	AI	Right	20°C, Partly Cloudy		PDB/MJD
:01	S							

:01	S						
:01	SR						
:01	SR						
:01	SR						
:01	SR						
:01	SR						
:01	SR						
:01	SR						
:01	SR						
:01	SR						
:01	SR						
:01	SR						
:14	SR						
30:00:00	PR (ends)						
Total Time			**Total Time**				
29.61 min			0				
							Day 20 June 23
0	PR	Left	:0	PR	Right	23.88°C, Partly Sunny	PDB/CKOB
:01	S		:14	PR (ends)			
:09	PR (ends)						
Total Time			**Total Time**				
9 secs			14 secs				
							Day 21 June 30
0-30:00min	AI	Right	0-30:00min	AI	Left	21.11°C, Cloudy	CKOM/OPIUM
Total Time			**Total Time**				
0			0				
							Day 22 July 7
0-30:00min	AI	Left	0-30:00min	AI	Right	30°C, Sunny	PRM/CKOB
Total Time			**Total Time**				
0			0				

							Day 23 July 14
0-30:00 min	AI	Right	0-30:00 min	AI	Left	28.88°C, Partly Sunny	PRM/OPIUM
Total Time			**Total Time**				
0			0				
							Day 24 July 28
:0	PR	Right	0-30:00 min	AI	Left	24.44°C, Sunny	PRM/MJD
:01	S						
:01	S						
:22							
Total Time			**Total Time**				
22 secs			0				
							Day 25 Aug. 4
0-30:00min	AI	Right	0-30:00min	AI	Left	25.55°C, Partly Sunny	CKOM/MJD
Total Time			**Total Time**				
0			0				
							Day 26 Aug. 11
0-30:00min	AI	Right	0-30:00min	AI	Left	25.55°C, Sunny	CKOM/CKOB
Total Time			**Total Time**				(note) exhibit change
0			0				
							Day 27 Aug. 18
0-30:00min	AI	Left	0-30:00min	AI	Right	26.66°C, Partly Sunny	CKOM/Opium
Total Time			**Total Time**				
0			0				

							Day 28 Sept. 15
0-30:00min	AI	Right	0-30:00min	AI	Left	22.77°C, Sunny	PDB/MJD
Total Time			**Total Time**				
0			0				
							Day 29 Oct. 06
0-30:00min	AI	Left	:00	PR	Right	20°C, Partly Sunny	PDB/Opium
			:01	S			
			:01	S			
			:01	L			
			:01	SR			
			1:10 min	PR (ends)			
			Total Time				
			70 secs				
							Day 30 Oct. 20
0-30:00min	AI	Right	0-30:00min	AI	Left	22.77°C, Sunny	PDB/CKOB
Total Time			**Total Time**				
0			0				
							Day 31 Oct. 27
0-30:00min	AI	Right	:00	PR	Left	10°C, Sunny	PRM/Opium
			:03	PR (ends)			
			:00	PR			
			:02	PR (ends)			
Total Time			**Total Time**				
0			5 secs				
							Day 32 Nov. 3
0-30:00min	AI	Right	0-30:00min	AI	Left	11.11°C, Sunny	PRM/CKOB

Total Time			**Total Time**					
0			0					
							Day 33 Nov. 10	
:00	PR	Left	0-30:00min	AI	Right	10°C, Sunny		PRM/MJD
:04	PR (ends)							
Total Time			**Total Time**					
4 secs			0					
							Day 34 Dec. 1	
:00	PR	Right	0-30:00min	AI	Left	10°C, Sunny		CKOM/CKOB
:01	S							
:01	S							
:04	PR (ends)							
Total Time			**Total Time**					
4 secs			0					
							Day 35 April 6	
0-30:00min	AI	Left	0-30:00min	AI	Right	12.77°C, Sunny		CKOM/MJD
Total Time			**Total Time**					
0			0					
							Day 36 April 13	
0-30:00min	AI	Right	0-30:00min	AI	Left	22.22°C, Partly Sunny		CKOM/Opium
Total Time			**Total Time**					
0			0					

Detailed data for every bout involving Shanto. Behavioral Codes are Active but Uninterested (AI), Proximity (PR), Flehmen (F), Sniff (S), Scent Roll (SR), Lick (L), Urinate (U), Bite (B), Paw (P), and Hiss (H). One failed day is included to describe why it was redone.

DATA SET STARTS FOR 2012

Time (min:sec) Male Fragrance	Behavior	Tree 1	Time (min:sec) Female Fragrance	Behavior	Tree 2	Weather	Date	Male/Female
							Day 1 Oct. 13	
0-30:00 min	AI	Right	0-30:00	AI	Left	18.88°C, Sunny		CKOM/CKOB
Total Time			**Total Time**					
0			0					
							Day 2 Oct. 20	
								CKOM/MJD
:00	PR	Left	:00	PR	Right	12.22°C, Partly Cloudy		
:02	S		:01	S				
:06	PR (ends)		:15	PR (ends)				
Total Time			**Total Time**					
6 secs			15secs					
							Day 3 Oct. 26	
								PDB/MJD
:00	PR	Right	0-30min	AI	Left	6.11°C, Partly Sunny		
:02	PR (ends)							
Total Time			**Total Time**					
2 secs			0					

							Day 4 Oct. 28
0-30:00min	AI	Left	0-30:00min	AI	Right	6.66°C, Sunny, Windy	PDB/CKOB
Total Time			**Total Time**				
0			0				
							Day 5 Nov. 2
							PRM/Opium
:00	PR	Right	:00	PR	Left	7.77°C, Partly Sunny	
:02	S		:08	PR (ends)			
:12	PR (ends)						
Total Time			**Total Time**				
12 secs			8 secs				
							Day 6 Nov. 3
							PRM/CKOB
0-30min	AI	Left	:00	PR	Right	9.44°C, Cloudy	
			:01	P			
			:02	S			
			:12	PR (ends)			
			:00	PR			
			:02	PR (ends)			
Total Time			**Total Time**				
0			14 secs				
							Day 7 Nov. 4
							PRM/MJD
0-30:00min	AI	Right	0-30:00min	AI	Left	6.66°C, Sunny	
Total Time			**Total Time**				
0			0				
							Day 8 Nov. 9
							CKOM/CKOB
0-30:00 min	AI	Left	:00	PR	Right	18.88°C, Sunny	
			:01	U			

			:15	PR (ends)			
Total Time			**Total Time**				
0			15 secs				
							Day 9 Nov. 10
							CKOM/MJD
0-30:00min	AI	Right	0-30:00min	AI	Left	17.77°C, Sunny	
Total Time			**Total Time**				
0			0				
							Day 10 Nov. 11
							CKOM/Opium
0-30:00min	AI	Right	0-30:00min	AI	Left	17.22°C, Cloudy	
Total Time			**Total Time**				
0			0				
							Day 11 Nov. 16
							PDB/MJD
:00	PR	Left	0-30:00 min	AI	Right	11.66°C, Sunny	
:04	PR (ends)						
Total Time			**Total Time**				
4 secs			0				
							Day 12 Nov. 17
							PDB/Opium
:00	PR	Right	0-30:00 min	AI	Left	11.11°C, Sunny	
:20	PR (ends)						
Total Time			**Total Time**				
20 secs			0				
							Day 13 Nov. 18
							PDB/CKOB
0-30:00min	AI	Left	0-30:00min	AI	Right	11.11°C, Sunny	

Total Time			**Total Time**				
0			0				
							Day 14 Nov. 30
0-30:00 min	AI	Left	0-30:00 min	AI	Right	14.44°C, Sunny	PRM/Opium
Total Time			**Total Time**				
0			0				
							Day 15 Dec. 1
0-30:00 min	AI	Right	0-30:00 min	AI	Left	14.44°C, Partly Sunny	PRM/CKOB
Total Time			**Total Time**				
0			0				
							Day 16 Dec. 2
0-30:00 min	AI	Left	0-30:00 min	AI	Right	20°C, Cloudy	PRM/MJD
Total Time			**Total Time**				
0			0				
				DATA SET STARTS FOR 2013-14			
							Day 17 May 5
0-30:00 min	AI	Left	0-30:00 min	AI	Right	21.66°C, Cloudy	CKOM/CKOB
Total Time			**Total Time**				
0			0				
							Day 18 May 19
0	AI	Left	0	AI	Right	28.33°C, Partly Sunny	PDB/OPIUM
Total			**Total Time**				

Time								
0			0					
							Day 19 June 09	
:00	PR	Right	0	AI	Left	23.33°C, Partly Sunny		CKOM/MJD
:07	PR (ends)							
Total Time			**Total Time**					
7 secs			0					
							Day 20 June 23	
0	AI	Left	0	AI	Right	25°C, Partly Sunny		CKOM/OPIUM
Total Time			**Total Time**					
0			0					
							Day 21 June 30	
:01	PR	Right	0-30:00 min	AI	Left	22.22°C, Cloudy		PDB/MJD
:04	PR (ends)							
Total Time			**Total Time**					
5 secs			0					
							Day 22 July 6	
0-30:00 min	AI	Right	0-30:00 min	AI	Left	28.88°C, Sunny		CKOM/Opium
Total Time			**Total Time**					
0			0					
							Day 23 July 14	
:00	PR	Right	0-30:00 min	AI	Left	28.88°C, Sunny		PDB/Opium
:03	U							
:01	S							

:12	PR (ends)							
Total Time			**Total Time**					
12 secs			0					
							Day 24 July 28	
0-30:00 min	AI	Left	:0	PR	Right	23.88°C, Sunny		PDB/CKOB
			:01	S				
			:08	PR (ends)				
Total Time			**Total Time**					
0			8 secs					
							Day 25 Aug. 4	
0-30:00 min	AI	Left	0-30:00 min	AI	Right	25.55°C, Partly Sunny		PRM/CKOB
Total Time			**Total Time**					
0			0					
							Day 26 Aug. 11	
0-30:00 min	AI	Right	0-30:00 min	AI	Left	27.77°C, Sunny		PRM/OPIUM
Total Time			**Total Time**					
0			0					
							Day 27 Aug. 25	
0-30:00 min	AI	Left	0-30:00 min	AI	Right	28.33°C, Sunny		PRM/MJD
Total Time			**Total Time**					
0			0					
							Day 28 Sept. 15	
0-30:00 min	AI	Right	0-30:00 min	AI	Left	22.22°C, Sunny		CKOM/CKOB
Total Time			**Total Time**					

0			0				
							Day 29 Sept. 29
0-30:00 min	AI	Left	0-30:00 min	AI	Right	20.55°C, Sunny	CKOM/MJD
Total Time			**Total Time**				
0			0				
							Day 30 Oct. 20
0-30:00 min	AI	Right	0-30:00 min	AI	Left	13.33°C, Sunny	CKOM/Opium
Total Time			**Total Time**				
0			0				
							Day 31 Oct. 27
0-30:00 min	AI	Right	0-30:00 min	AI	Left	10°C, Sunny	PDB/MJD
Total Time			**Total Time**				
0			0				
							Day 32 Nov. 3
0-30:00 min	AI	Left	0-30:00 min	AI	Right	10°C, Sunny	PDB/Opium
Total Time			**Total Time**				
0			0				
							Day 33 Nov. 10
0-30:00 min	AI	Right	:00	PR	Left	10°C, Sunny	PDB/CKOB
			:01	S			
			:01	U			
			:03	PR (ends)			
Total Time			**Total Time**				

0			3 secs					
							Day 34 Dec. 1	
:00	PR	Right	:00-30:00 min	AI	Left	10°C, Sunny		PRM/Opium
:03	S							
:01	U							
1:03 min	PR (ends)							
:00	PR							
:04	PR (ends)							
Total Time			**Total Time**					
1:07 min			0					
							Day 35 April 6	
0-30:00 min	AI	Left	:00	PR	Right	11.11°C, Partly Sunny		PRM/CKOB
			:02	PR (ends)				
Total Time			**Total Time**					
0			2 secs					
							Day 36 April 13	
:0	PR	Left	0-30:00 min	AI	Right	10°C, Sunny		PRM/MJD
:03	PR (ends)							
Total Time			**Total Time**					
3 secs			0					

APPENDIX B

INDIVIDUALITY OF CHEETAH BEHAVIOR

Jason

Jason was three years old when the study began (Figure 16). Jason was the most active of the four cheetahs. He had several patrol patterns, and usually sniffed at the scent dispensers before interacting with them any further. Urinating was his first reaction when given the male musky scent as enrichment in one case. Jason was also the only cheetah to display scent rolling behavior while interacting with the scent dispensers.

Zuri

Zuri was born in the St. Louis Zoo and was five years old when the study began (Figure 17). Zuri was interested in the scent dispensers and displayed a few behaviors the other cheetahs did not, but did not urinate in response to the enrichments. Zuri displayed flehmen behavior, but the scent dispensers were unlikely to be the cause of her response because there was a strong odor of elephant feces and she was not near the dispensers. Zuri attempted to move dispensers three different occasions by picking them up with her mouth or paw. Zuri responded to olfactory enrichments by first sniffing or pawing at them. Playful stalking or charging behavior was also recorded. At times Zuri spent more time observing the Asian elephants, and infrequently interacted with her brother Kgosi.

Shanto

Shanto was the least active of the three cheetahs that interacted with the scent dispensers, but the only cheetah besides Jason to urinate during the bouts. Shanto kept similar patrol patterns in the exhibit, but changed them occasionally (Figure 18). Even during his changed patterns Shanto would approach the scent dispensers even if he did not see them, and then interact with them. Shanto’s first response was usually to sniff, but a few times his first behavioral response was to urinate before interacting with the scent dispensers.

Kgosi

Kgosi was born in the St. Louis Zoo and was five years old when the study began (Figure 19). Kgosi did not interact with the fragrances. Most of his activity involved watching gray squirrels, watching the train, or communicating with Zuri his sister. On two occasions Kgosi raised up on his exhibit fencing to watch Zuri interact with the scent dispensers, but when presented with his own he merely watched them get placed in his exhibit.

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Major Professor: Carey Krajewski